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ADDENDUM TO

HEALTH IMPLICATIONS OF EXPOSURE OF UNDERGROUND MINE WORKERS TO DIESEL EXHAUST EMISSIONS

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TABLE OF CONTENTS

	Page Number
1. INTRODUCTION	1
2. RECENT EMISSION DATA	5
3. EFFECTS OF EXHAUST PURIFIERS ON EMISSIONS	18
4. MUTAGENICITY	24
4.1 Mutagenicity Results from Bacterial Cell Systems	24
4.2 Mutagenicity Results from Mammalian Cell Systems	36
5. DEPOSITION AND CLEARANCE OF INHALED SUBSTANCES	39
6. WHOLE ANIMAL, INHALATION STUDIES	50
6.1 Health Effects of Specific Exhaust Fractions	50
6.1.1 Health Effects of Filtered and Unfiltered Emissions	50
6.1.2 Health Effects of Inert Particles	69
6.1.3 Health Effects of PAH-Enriched Emissions	77
6.2 Health Effects of Total Exhaust	83
6.2.1 Review Article	83
6.2.2 Exposure to Total Exhaust Coupled with Carcinogenic Pretreatment	89
6.2.3 Exposure to Total Exhaust without Pretreatment	95
7. WHOLE ANIMAL, NON-INHALATION STUDIES	104
8. EPIDEMIOLOGY	108
9. LIMITATIONS OF RESEARCH	112
10. HUMAN HEALTH IMPLICATIONS	115
10.1 Anatomical Pathology of Tumours and Cancers	115
10.1.1 Locations of Lung Tumours and Cancers	115
10.1.1.1 Human Pathology	115
10.1.1.2 Metaplasia	116
10.1.1.3 Animal Pathology	117
10.2 Cell Types in Human Lung Tumours and Cancers	117
10.2.1 Cellular Types in Humans	117
10.2.2 Review of Animal Tumours	118
10.3 Hypotheses of Cellular Development to Malignant Tumours	118
10.3.1 Squamous Cell Lung Cancer Development	118
10.3.2 Metastases	119
10.3.3 Multiple Factors Leading to Cancer	120
10.3.3.1 Fractions of Exhaust and Health Effects	121
10.3.4 Development of Human Related Research and Treatment	125
10.4 Diesel Exhaust Health Effects in Humans	126
10.4.1 Total Effect	126
10.4.1.1 Scandanavian Workers Exposed to Total Diesel Exhaust	126

.../cont'd

TABLE OF CONTENTS (Cont'd)

	Page Number
10.4.2 Pulmonary Function Deficits	127
10.4.2.1 Obstructive Defect	127
10.4.2.2 Restrictive Defect	127
10.4.2.3 Mixed Deficits	127
10.4.2.4 Research Measurements	128
10.4.3 Diesel Exhaust and Lung Cancer in Railroad Workers	128
10.5 Personal Lifestyle of the Occupationally-Exposed Worker	129
10.5.1 Alcohol Use	129
10.5.2 Cigarette Smoking	129
10.5.2.1 Impairment of Lung Clearance	129
10.5.2.2 Effect of Smoking on Respiratory Symptoms	130
10.5.2.3 The Effect of Cigarette Smoking on Natural Killer Cells - A Major Defence System	130
11. SUMMARY	132
12. CONCLUSIONS	138
13. BIBLIOGRAPHY	142

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1. INTRODUCTION

In April, 1984 French and Mildon published an update to their 1979 review of the world literature on the health implications of exposure of underground mine workers to diesel exhaust(1). Since that time a number of additional relevant toxicity, carcino-genicity and mutagenicity trials have been reported in the literature. In addition, a considerable volume of analytical data on the polycyclic aromatic content of diesel exhaust and their identification as possible carcinogenic components of exhaust has been reported. The Department of Energy Mines and Resources of Canada have issued a contract to review the details of these newly published studies and to evaluate their significance to the potential health effects of diesel exhaust on underground mine workers. In particular, EMR requested a revision of the air quality index (AQI) and give consideration to modification in light of newly available scientific data. The AQI was proposed as a means of estimating the relative potential for diesel exhaust pollutants to adversely affect the health of miners. Although at the time of the previous reports there was little data available to demonstrate an increased risk of lung cancer as a result of diesel exhaust exposure, the authors concluded that, of the diesel exhaust pollutants evaluated, particulate had the most significant potential for adverse health effects. Therefore, the AQI equation was weighted towards respirable, combustible particulate and appeared in the equation three times, as follows:

It was suggested that two equations be used, as follows:

$$\textit{AQI (gas):} \quad \frac{[CO]}{TLV_{CO}} + \frac{[NO]}{TLV_{NO}} + \frac{[NO_2]}{TLV_{NO_2}}$$

It was recommended that the AQI (gas) should not exceed 1, and no individual component should exceed its TLV.

$$\textit{AQI (particulate):} \quad \frac{[RCD]}{TLV_{RCD}} + \left[\frac{[SO_2]}{TLV_{SO_2}} + \frac{[RCD]}{TLV_{RCD}}\right] + \left[\frac{[NO_2]}{TLV_{NO_2}} + \frac{[RCD]}{TLV_{RCD}}\right]$$

(Source: Reference 1)

Furthermore, it was recommended that the AQI (particulate) value should not exceed 2.0, and no single term should exceed its TLV, as dictated by current ACGIH standards.

Before discussing the toxicity data, which are the main thrust of the present update, it is important to review the authors original contention that "for most carcinogenic chemicals, there is a dose below which the development of cancer is not likely to occur and/or there is a dose below which the risk of developing cancer is sufficiently low as to be acceptable to society"(1, p.37). At the time of the 1984 report, the preponderant view

of toxicologists was that any dose of a carcinogen was unacceptable. In 1987 the US Food and Drug Administration (FDA) published a final rule setting criteria and procedures for evaluating the safety of carcinogenic residues(2). This has resulted in a revision of the Delaney regulation which held that no concentration of a potentially carcinogenic compound would be allowed as a food additive. The new regulation, which supports the authors view of a dose-response relationship for carcinogenic compounds, provides for the application of a statistical extrapolation of animal carcinogenicity data from which the concentration of carcinogenic residues of concern corresponds to a maximum lifetime risk to the test animal of 1 in 1 million*.

The FDA stated that "such a concentration of residue will be considered safe and will be permitted in the total diet of people". The FDA emphasized that the one in one million level of risk did not mean that one in one million people would contract cancer, but that (a) it was a one in one million increase in risk over the normal risk of cancer to the test animal, and (b) it is a lifetime, not annual, risk factor. Table 1 provides a calculation of the estimated "safe" doses for risks of less than one in a million based on the observed proportion of animals with tumours in a carcinogenicity trial(2).

Now that a regulatory agency has promulgated a quantitative assessment of risk relative to carcinogenic compounds in the food supply, a similar rationale may be appropriate when considering the potential carcinogenicity of diesel exhaust.

Of the experiments conducted in recent years, most have concentrated on the effects of particulate and polycyclic aromatic hydrocarbon (PAH) components of diesel emissions. This polarization of energy is hardly surprising considering the fact that the particulate fraction of diesel exhaust has been identified as the largest single contributor to the toxicity of raw exhaust(1,p.330).

Much of the toxicity of exhaust gases was known a decade ago which, at worst, indicated that the gaseous pollutants were irritants. In contrast, some of the PAH's present in diesel soot were known mutagens, and, therefore potential carcinogens. Relatively little was known of the extent to which these PAH's and the particulate to which they attached represented a health risk. Consequently, that these exhaust fractions should almost monopolize the diesel research community's attention is understandable.

(*) In studies cited by Paustenbach(3), the risks taken voluntarily would be in the range of one in one thousand. On the other hand, the imposition of a fatality risk on a person, without his or her knowledge is considered inappropriate by some, including several governmental agencies, if the risk is greater than one in one million. This is the level of risk that was adopted by the Food and Drug Administration and that was cited above. Still, according to Paustenbach(3), potentially fatal, involuntarily risks as high as one in ten thousand may be imposed as long as the exposed group derives significant benefit from the activity.

Table 1
Observed Proportion of Animals with Tumours and Calculation of Estimated "Safe" Doses for Risks of Less than One in a Million
(Source: Reference 2)

Doses	Proportion of Animals with Tumours							Upper 95% Confidence Limit at Lowest Dose[1]	Estimated "Safe" Dose[2]
	0	0.01	0.05	0.1	0.2	0.5	1.0		
Linear	0/50	---	---	---	---	5/50	10/50	0.150	3.3×10^{-6}
	0/50	---	---	---	2/50	5/50	10/50	0.061	3.3×10^{-6}
	0/50	---	---	1/50	2/50	5/50	10/50	0.031	3.3×10^{-6}
	0/50	---	0/50	1/50	2/50	5/50	10/50	0.015	3.4×10^{-6}
	0/50	0/50	0/50	1/50	2/50	5/50	10/50	0.003	3.4×10^{-6}
Curved	0/50	---	---	---	---	2/50	10/50	0.105	3.3×10^{-6}
	0/50	---	---	---	0/50	2/50	10/50	0.024	3.3×10^{-6}
	0/50	---	---	0/50	0/50	2/50	10/50	0.010	3.3×10^{-6}
	0/50	---	0/50	0/50	0/50	2/50	10/50	0.004	3.4×10^{-6}
	0/50	0/50	0/50	0/50	0/50	2/50	10/50	0.001	3.4×10^{-6}

1 Upper 95% confidence limits were obtained by fitting the multistage model to the data by the procedure given by Howe, R.B. and Crump, K.S. *Global 82: A Computer Program to Extrapolate Quantal Animal Toxicity Data to Low Doses*, K.S. Crump and Co., Inc., Ruston, LA.

2 The estimated "safe" dose is obtained by linear extrapolation from the upper confidence limit at the lowest experimental dose to zero. For a maximum risk of one in a million, "safe" dose = lowest experimental dose $\times 10^{-6}$/upper confidence limit.

In the course of this research, two mechanisms have emerged that each partially describe the health effects of diesel emissions.

On one hand, the organic compounds associated with diesel particulate could effect mutations in critical sites of the genome of a somatic cell and transformation of exposed cells to an anaplastic state may result. On the other hand, the regenerative cellular proliferation that is observed in response to cytotoxicity by chemicals or by large accumulations of particles in the lungs could be classified as epigenetic, because it would not involve any direct interaction with the genome(4, 5). According to the latter mechanism, adverse health effects are experienced only after exposure to a certain threshold dose. With the former, there is an assumption that a single molecule could initiate atypical proliferation. Vostal(6) described these mechanisms of toxicity as shown below (Figure 1).

Both mechanisms have met some opposition because, on their own, they have failed to fully explain the development of anaplasia. Stott and Watanabe(5) discussed the details of these views and included alternative theories. Their discussion should be consulted for more details.

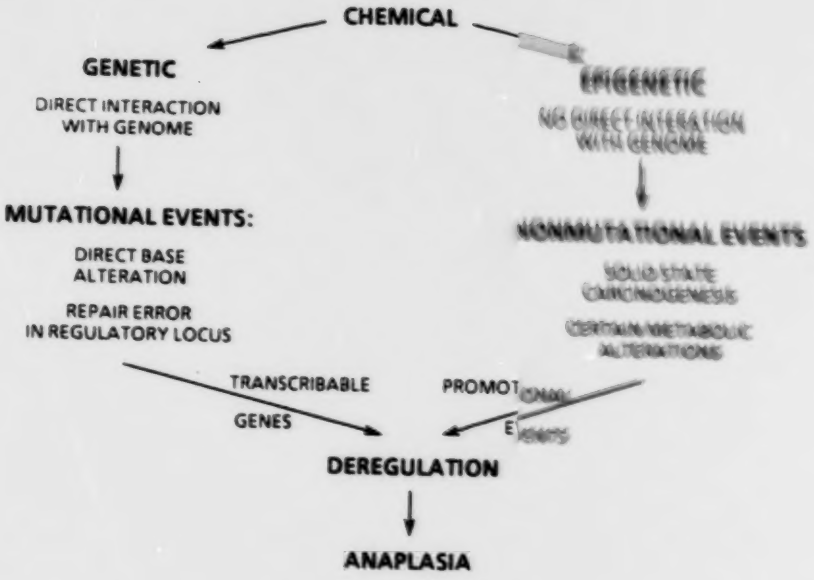


Figure 1

Schematic Differentiation of Genetic Versus Epigenetic Mechanisms of Carcinogenicity

(Source: Reference 6)

In 1986, Lewtas and Williams[7] recognized that many questions remained relating to the toxicity of diesel engine emissions. They acknowledged the problem, even though they admitted that substantial advances had been made since 1978 when compounds extracted from diesel engine particulate were found to be mutagenic.

Of the numerous studies that have been completed in the last few years, those from a few research centres have consistently been at the leading edge of this complex field. The results of the experiments conducted at these facilities have gained much importance because of their thoroughness. For this reason, results from these research laboratories will be emphasized in this report.

These centres include:

- Batelle, Geneva, Switzerland
- Fraunhofer Institute fur Toxikologie und Aerosolforschung, Hannover, West Germany
- General Motors Research Centre, Warren, Michigan, USA
- Japan Automobile Research Institute Inc., Japan
- Japanese Research Institute of Tuberculosis, Tokyo, Japan
- Lovelace Biomedical and Environmental Research Institute, Albuquerque, New Mexico, USA
- Saitama Medical School's Department of Public Heath, Saitama, Japan

8. RECENT EMISSION DATA

The characterization and quantitation of the components of diesel exhaust has been the object of much activity. This section will detail the emission measurements that have been published in the last few years. Hopefully they will allow a determination as to whether or not current emission limits are being exceeded. Also, these measurements, together with mutagenicity and toxicity data should help to decide whether or not new standards are required.

A few researchers have written on the facilities and experimental procedures available or used to obtain the results that are the subject of the present discussion. However, these descriptions are not summarized here. They can be found in References 8, 9 and 10 which should be consulted for any detail. Also, for an extensive description of diesel engine structure and mechanics the reader is referred to the 1984 edition of this report(1).

Lies et al(11) found that a 1.5 litre Rabbit-D 1978, 4 cylinder diesel engine had the highest particulate emission when run through the Federal Test Procedure (FTP) cycle, as compared to the Fuel Economy Test (FET) or the Sulphate Emission Test (SET). Vehicle particulate emissions ranged between 225 and 325 mg/mile, and thus it is reasonable to accept an average particulate emission of 300 mg/mile. PAH output from the 1978 Rabbit-D, 1.5 VW diesel engine was provided by Kraft and Lies(12). Figure 2 provides the details of the PAH distribution in the exhaust of the Rabbit D engine.

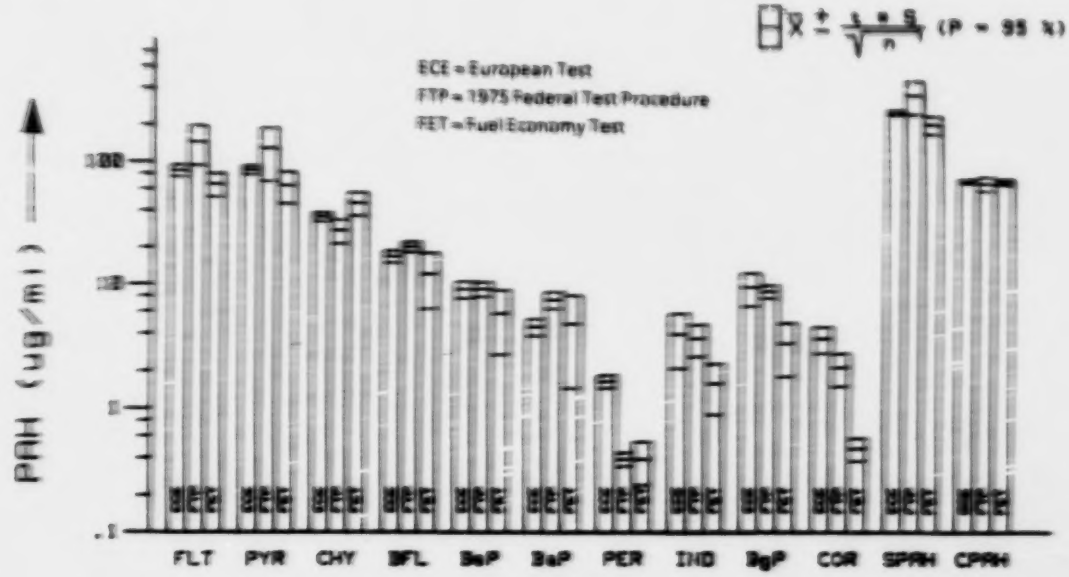


Figure 2

Exhaust PAH Emissions of a Rabbit D (1978)
(Source: Reference 12)

The PAH and benzo(a)pyrene (BaP) content of emission from the Rabbit D engine are shown in Figures 3 and 4, respectively.

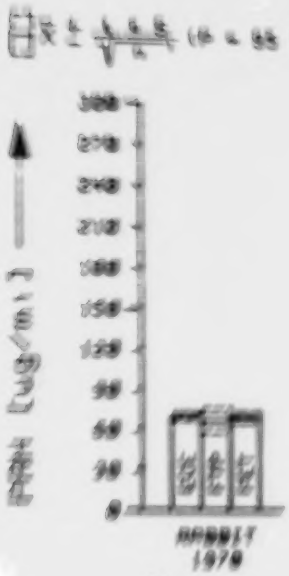


Figure 3

Exhaust PAH Emissions of a 1978 VW Rabbit Equipped with a Diesel Engine
(Source: Reference 12)

Figure 4

Exhaust BaP Emissions of a 1978 VW Rabbit Equipped with a Diesel Engine
(Source: Reference 12)

The SPAH column in Figure 2 refers to the sum of the PAH's and the CPAH column to the portion of the biologically active compounds. Biological activity was assessed as indicated in Table 2.

Additional characteristics of the emissions output of the 1.5 litre VW and 2.4 litre Mercedes Benz engines were presented by Hare and Baines[13]. Speed-time traces for the FTP and FET operating cycles are shown in Figure 5.

The average speed for the FTP cycle was 31.5 km/hr and for the FET 77.5 km/hr. The gaseous emissions from the two engines are summarized in Table 3, which also includes the emission values for a driving schedule of 50 and 85 kph.

Data on the particulate and other emission characteristics for the two engines during the FTP, FET, 50 and 85 kph schedules are provided in Tables 4 and 5 for the Mercedes 240D and the VW Rabbit, respectively.

Table 2

Polycyclic Aromatic Hydrocarbons, 4-6 Rings
(Source: Reference 16)

Name PAH	Formula	M.W.*	B.P. (°C)**	Biological Activity***
Fluoranthene	$C_{16}H_{10}$	202	384	-
Pyrene	$C_{16}H_{10}$	202	394	-
Benzo(ghi)fluoranthene	$C_{18}H_{10}$	226	-	-
Benz(a)anthracene	$C_{18}H_{12}$	228	438	+
Chrysene	$C_{18}H_{12}$	228	448	+
Benzo(k)fluoranthene	$C_{20}H_{12}$	252	481	-
Benzo(b)fluoranthene	$C_{20}H_{12}$	252	481	+ +
Benzo(a)pyrene	$C_{20}H_{12}$	252	496	+ + +
Benzo(e)pyrene	$C_{20}H_{12}$	252	493	+
Perylene	$C_{20}H_{12}$	252	497	?
Benzo(ghi)perylene	$C_{22}H_{12}$	276	500	?
Indeno (1,2,3-cd)pyrene	$C_{22}H_{12}$	276	534	+
Anthanthene	$C_{22}H_{12}$	276	547	?
Coronene	$C_{24}H_{12}$	300	602	?

(*) M.W. = Molecular Weight
(**) B.P. = Boiling Point
(***) Animal skin painting [+ + + very strong; + + strong; + weak; - none]

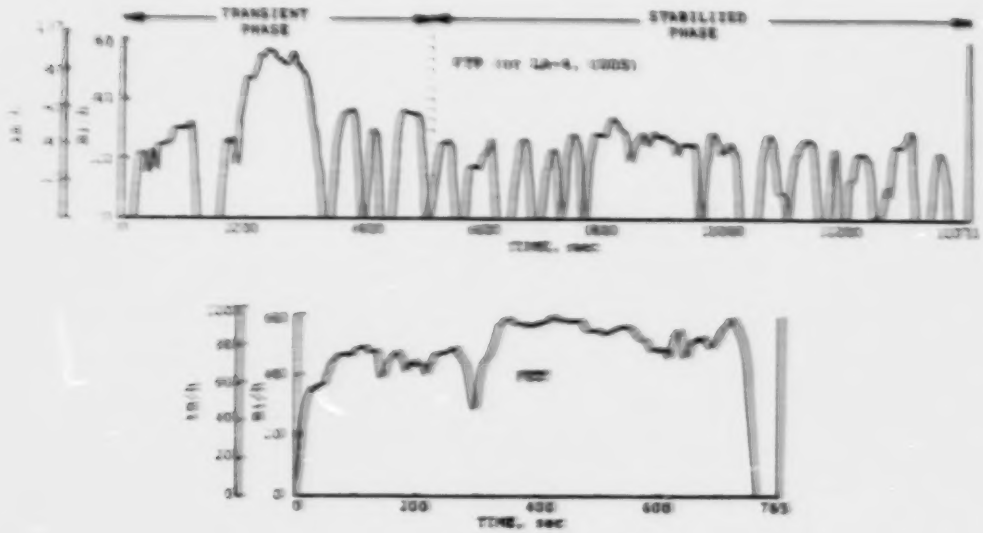


Figure 5

Speed-time Traces of FTP and FET Operating Schedules
(Source: Reference 13)

Table 3

Summary of Routine Gaseous Emissions Results Using EM-238-F "2D Emissions" Test Fuel

(Source: Reference 13)

Vehicle	*Driving Schedule*	*HC,[a] g/km*	*CO,[a] g/km*	*CO_2,[a] g/km*	*NO_X,[a] g/km*	*Fuel,[a] L/100 km*
Mercedes 240D	1975 FTP	0.12	0.57	226	0.78	8.48
	FET	0.06	0.35	172	0.68	6.43
	50 kph	0.08	0.27	124	0.47	4.64
	85 kph	0.08	0.36	172	0.84	6.44
VW Rabbit Diesel	1975 FTP	0.16	0.49	153	0.59	5.71
	FET	0.11	0.32	116	0.53	4.35
	50 kph	0.10	0.27	92	0.31	3.44
	85 kph	0.08	0.33	119	0.55	4.45

[a] Idle emissions in g/h instead of g/km, fuel in L/h instead of L/100 km

Comparison of the data from each vehicle indicates that mean particulate mass rates, mean sulphur and sulphate, and mean values of gaseous emissions (HC,CO,NO_X) by operating schedule agreed quite well for the two engines. However, rank ordering of solubles as percent of particulate, of BaP and of mean values for percent of particulate not identified as carbon, hydrogen, nitrogen or sulphur did not show good agreement between vehicles. The degree of variability present indicates that these trends cannot be generalized safely to include other vehicles.

In general, it was found that sulphur and sulphate emissions were well correlated with fuel sulphur content, that over 85% by weight of particulate material had an aerodynamic diameter under 0.4 μm; that soluble organic in particulate did not account for more than 16% by weight of particulate, that solubles are mainly hydrocarbons with impurities and substituted groups; and that emission differences between vehicles and operating procedures were greater than those between fuels.

A related series of tests by Gibbs et al(15) generated data that are noticeably different from those presented above. In their two year study twenty in-use diesel automobiles were tested to characterize emission data with major emphasis on particulate and its contents. The percentage of soluble organic fraction (SOF) in total particulate is compared for different driving cycles in Tables 4, 5 and 6.

Table 4

Multiple Comparisons of Mean Emission Values, Mercedes 240D

(Source: Reference 13)

Values Listed in Inverse Rank Order

Particulate (g/h)				*Solubles (% of Particulate)*				*Sulphur (mg/h)*			
Operating Schedule	*Mean[a] Value*	*Fuel*	*Mean[a] Value*	*Operating Schedule*	*Mean[a] Value*	*Fuel*	*Mean[a] Value*	*Operating Schedule*	*Mean[a] Value*	*Fuel*	*Mean[a] Value*
50 km/h	6.87	EM-239-F	9.77	FET	6.54	EM-241-F	5.67	50 km/h	(47.50)	EM-238-F	(137.98)
1975 FTP	9.75	EM-241-F	12.31	85 km/h	7.40	EM-242-F	6.53	1975 FTP	(91.80)	EM-242-F	152.95
FET	15.24			50 km/h	8.78	EM-238-F	9.57	FET	188.84		
85 km/h	15.69			1975 FTP	9.90			85 km/h	236.30		
Sulphate (mg/h)				*BaP (µg/h)*				*(100-CHNS) (% of Particulate)*			
Operating Schedule	*Mean[a] Value*	*Fuel*	*Mean[a] Value*	*Operating Schedule*	*Mean[a] Value*	*Fuel*	*Mean[a] Value*	*Operating Schedule*	*Mean[a] Value*	*Fuel*	*Mean[a] Value*
50 km/h	(112.10)	EM-242-F	332.62	85 km/h	5.36	EM-242-F	8.29	50 km/h	15.46	EM-239-F	16.38
1975 FTP	226.20	EM-238-F	395.08	FET	(8.36)	EM-240-F	(9.43)	FET	(19.21)	EM-241-F	19.88
85 km/h	601.80			50 km/h	(8.38)	EM-238-F	(10.76)	85 km/h	(19.60)	EM-242-F	24.14
FET	668.22			1975 FTP	(12.70)			1975 FTP	(20.48)		
Gaseous HC (g/h)				*CO (g/h)*				*NO_X (g/h)*			
Operating Schedule	*Mean[a] Value*	*Fuel*	*Mean[a] Value*	*Operating Schedule*	*Mean[a] Value*	*Fuel*	*Mean[a] Value*	*Operating Schedule*	*Mean[a] Value*	*Fuel*	*Mean[a] Value*
50 km/h	(3.00)	EM-238-F	(4.08)	50 km/h	13.40	EM-240-F	(19.90)	50 km/h	23.20	EM-239-F	(31.44)
FET	(4.03)	EM-241-F	4.64	1975 FTP	19.95	EM-241-F	22.16	1975 FTP	25.36	EM-241-F	33.14
1975 FTP	(4.53)			FET	30.54			FET	55.66		
85 km/h	(5.10)			85 km/h	33.49			85 km/h	62.73		

[a] Brackets surrounding means *not* significantly different at the 0.05 level

Table 5

Multiple Comparisons of Mean Emission Values, VW Rabbit Diesel

(Source: Reference 13)

Values Listed in Inverse Rank Order

Particulate (g/h)				Solubles (% of Particulate)				Sulphur (mg/h)			
Operating Schedule	Mean[a] Value	Fuel	Mean[a] Value	Operating Schedule	Mean[a] Value	Fuel	Mean[a] Value	Operating Schedule	Mean[a] Value	Fuel	Mean[a] Value
50 km/h	(4.54)	EM-239-F	7.25	FET	(13.40)	EM-242-F	12.74	50 km/h	12.70	EM-240-F	16.26
1975 FTP	(7.48)	EM-241-F	10.74	1975 FTP	(13.68)	EM-240-F	15.83	1975 FTP	53.10	EM-238-F	89.46
FET	(12.45)			85 km/h	(13.82)	EM-241-F	16.43	FET	101.71		
85 km/h	13.11			50 km/h	(17.00)			85 km/h	131.58		

Sulphate (mg/h)				BaP (µg/h)				(100-CHNS) (% of Particulate)			
Operating Schedule	Mean[a] Value	Fuel	Mean[a] Value	Operating Schedule	Mean[a] Value	Fuel	Mean[a] Value	Operating Schedule	Mean[a] Value	Fuel	Mean[a] Value
50 km/h	49.20	EM-240-F	70.24	50 km/h	9.65	EM-238-F	25.01	85 km/h	16.94	EM-239-F	26.68
1975 FTP	(162.52)	EM-238-F	266.58	85 km/h	(27.71)	EM-241-F	55.08	FET	24.76	EM-240-F	(31.74)
85 km/h	380.80			FET	(35.97)			1975 FTP	24.95	EM-241-F	32.95
FET	386.05			1975 FTP	52.39			50 km/h	31.88		

Gaseous HC (g/h)				CO (g/h)				NO_X (g/h)			
Operating Schedule	Mean[a] Value	Fuel	Mean[a] Value	Operating Schedule	Mean[a] Value	Fuel	Mean[a] Value	Operating Schedule	Mean[a] Value	Fuel	Mean[a] Value
50 km/h	3.60	EM-242-F	5.99	1975 FTP	(18.12)	EM-240-F	(21.04)	50 km/h	16.40	EM-239-F	23.68
1975 FTP	(9.19)			50 km/h	(19.00)	EM-241-F	27.87	1975 FTP	19.00	EM-242-F	24.61
FET	(9.61)			FET	(29.30)			FET	41.55		
85 km/h	(10.03)			85 km/h	31.11			85 km/h	52.53		

[a] Brackets surrounding means *not* significantly different at the 0.05 level

Table 6

Percentage of SOF From Exhaust of Two Samples of Diesel Automobiles for Different Testing Procedures
(Source: Reference 15)

Engine	*FTP*	*FHET*	*50 mg Cruise*	*Sample (n)*
VW	20.0	21.5	20.7	36
MB	14.0	13.6	15.2	18

In general, the percentage of SOF was less for the FTP than the other cycles. In a separate test, it was found that the VW engine emitted 0.34g/m3 of particulate in the FTP cycle.

Williams and Chock(16) provided useful observations on exhaust contents of extractables and of benzo(a) pyrene. Their results indicated that high molecular weight hydrocarbons (greater than twelve carbons) are deposited on diesel particles before entering the atmosphere. Also, their reactivity is low enough not to impact significantly on photochemical ozone formation. Such photochemical reactions would not take place in underground environments, but knowledge of the relative stability of these compounds may be helpful when attempting to predict the overall risk to exposed workers.

In the case of BaP it was found that the quantity of the compound tended to increase with exhaust dilution (Table 7). To explain this observation, it was postulated that in more concentrated exhaust BAP would be converted to other products, probably by reaction with NO_2. According to the authors, the experiments provided reliable evidence for the binding of the compound to the particles as they leave the tailpipe and, for the possibility that BaP remains associated with particulate matter, at least through the initial dilution phase.

Additional articles, cited below, provide additional examinations of the PAH content of diesel exhaust. Table 8(17) gives a list of some of the compounds and classes of compounds that can be found in diesel emissions.

In a study by Hakala et al(14) measured the concentration of polycyclic aromatic hydrocarbons (PAH's) in the atmosphere of a Finnish mine emitted from diesel engines of unspecified types. Only particulate fraction was collected because in cooled and diluted exhaust gas, most PAH's are condensed on the diesel soot and/or on the dust of ambient air, especially the carcinogenic PAH's including benzo(a)pyrene (BaP). The samples were taken from both the breathing zone of engine drivers and near the driveways of diesel engines. Altogether, 15 PAH compounds were determined. The

concentrations of total PAH and BaP in air and the percentages of BaP are shown in Table 9.

Table 7

The Effect of Dilution Ratio On Particulate Mass Concentration and Composition

(Source: Reference 16)

	Dilution Ratio	*No. of Samples*	*Specific Particulate Concentration (µg/m³ particulate/ ppm of CO_2)*	*Extractables (%)*	*BaP (ng/mg)*
Open Air	<2	1	--[a]	15	5
	5.1 - 7.0	5	5.0	26	19
	11 - 17	4	4.1	28	22
	21 - 24	3	3.5	28	28
	44 - 88	4	4.5	29	25
	135 - 150	2	6.5	28	32
	220 - 350	2	3.7	--[b]	20[c]
Dilution Tunnel	13.8	1	3.9	27	23
	26.5	1	3.8	27	22

[a] CO_2 concentration was above instrument capability
[b] Extractable mass was too small for accurate determination
[c] Large background correction to mass makes this number uncertain

The results show that the concentrations of BaP in the mine atmosphere were low. Also, the concetnrations of total PAH's were low. The relative concentration of BaP to the total PAH concentrations were on average 3.4% (SD ± 1.1).

Schuetzle[18] provided an extensive list of exhaust component concentrations (Table 10).

These data will be useful when trying to determine whether or not the concentrations of the various emitted components exceed the accepted maximal values.

Zierock et al[19] discussed findings on the exhaust polycyclic aromatic hydro-carbon (PAH) content of three diesel engines. Two of the three engines were production series engines and one was a prototype (Table 11).

Table 8

Selected Organic Compounds Identified From Diesel Particulate Matter's Soluble Organic Fraction (SOF)

(Source: Reference 17)

Paraffinic Compounds	*Aromatic Compounds*	*Acidic Compounds*
Octane	Ethyl naphthalene	Cresol
n-Tetradecane	Phenanthrene	Phenylphenols
n-Hexadecane	Fluoranthene*	Naphthols
n-Eicosane	Benzo(a)pyrene*	Naphtholic acids
Cyclohexane	Methyl phenanthrene*	Benzoic acids
Methyl cyclohexane		
Basic Compounds	*Transitional Compounds*	*Oxygenated Compounds*
Benzacridine	1-Nitropyrene*	9,10-Anthraquinone
Methyl aniline	9-Fluorenone	Hydroquinone
o-Toluidene	2-Methyl-9-anthrone	Benzanthrone
Dibenzo(c,g)carbazole	1,8-Naphthalic acid anhydride	Dinitro-o-cresol

Table 9

Concentration of Particulates, Total-PAH and BaP in the Mine Atmopshere

(Source: Reference14)

Sample	*Particulates (mg/m^3)*	*Total-PAH (ng/m^3)*	*BaP (ng/m^3)*	*BaP/Total-PAH (%)*
1	2.0	408	17	4.2
2	0.9	226	9	4.0
3	2.5	181	7	3.9
4	2.7	470	18	3.8
5	1.0	244	8	3.3
6	4.0	581	21	3.6
7	7.7	443	13	2.9
8	6.2	538	21	3.9
9	27	426	6	1.4
10	19	673	36	5.3
11	27	801	12	1.5
12	13	569	18	3.2

Parameter	HDD	LDD
Gas-phase (g/mi)		
CO	10 (36,23) 8 (35)	3 (37) --
NO_X	28 (35)	1 (37)
Gas-phase (mg/mi)		
Formaldehyde	--	20 (39)
Propylene	--	
Benzene	--	24 (39)
Toluene	11 (40)	--
Gas-phase (µg/mi)		
2-Nitrofluorene	--	90 (7)
Anthracene	8960 (37)	2100 (37)
Pyrene	1580 (37) 1580 (41)	380 (37) 1130 (41)
Fluoranthene	1240 (37) --	300 (37) 910 (7)
Particle-phase (µg/mi)		
Anthracene	439 (37)	105 (37)
Pyrene	1182 (37) -- -- --	284 (37) 848 (7) 284 (41) 39*
Fluoranthene	933 (37) -- --	224 (37) 683 (7) 933 (41)
2-Nitrofluorene	--	97 (7)
1-Nitropyrene	45 (37) --	11 (37) 4*
Benzo(a)pyrene	54 (37) -- -- -- 142 (41)	13 (37) 1* 3 (41) -- 34 (41)
Benzo(e)pyrene	64 (37) --	15 (37) 3*
Other Emissions		
Total Part. (mg/mi)	1660 (7) 1490 (35)	394 (7) 607 (35)
Total Ext. (mg/mi)	301 (7) 335 (35)	198 (7) 124 (35)
Mutagenicity** (rev./mi/10 + 6)		
TA98 (-S9)	0.37 (7)	0.95(7) 0.99*
TA98 (+ S9)	0.33 (7) --	0.36 (7) 0.59*

Table 10

Summary of Emission Levels From Current (1980 - 1985) Diesel Engines (FTP Cycle Only)

(Source: Reference 18)

* Table 3, Ref. 18 (22% fuel aromaticity)

** Mutagenicity values given in Table 8 of Ref. 7 in Ref. 18 of this report should be given as rev/km x 10-3 instead of rev/km

Table 11

Performance Specifications of the Engine Under Investigation
(Source: Reference 19)

	Engine A	*Engine B*	*Engine C*
Combustion System	Swirlchamber (WK)	Direct Injection (DE)	Prechamber (VK)
Output power at rpm (kW/(min)$^{-1}$)	36.6/5000	66.0/3000	59.0/4400
Number of cylinders	4	4	5
Displacement (dm^3)	1.47	3.79	3.0
Compression ratio	1:23	1:18.1	1:20. . .22

The fuel used contained 0.31% sulphur and the engines were operated in about 20-steady state modes at various loads and speeds. The exhaust emissions were diluted in filtered air according to a range of ratios that varied between 1:20 and 1:250 (Table 12), which gives the compounds analyzed.

Table 12

Analyzed PAH Compounds
(Source: Reference 19)

PAH Compound	*Formula*	*Molecular Weight*	*Abbreviation*
Fluoranthene	$C_{16}H_{10}$	202	FL
Pyrene	$C_{16}H_{10}$	202	Py
Benz(e)pyrene	$C_{20}H_{12}$	252	BeP
Benz(b)fluoranthene	$C_{20}H_{12}$	252	BbF
Perylene	$C_{20}H_{12}$	252	Per
Benz(k)fluoranthene	$C_{20}H_{12}$	252	BkF
Dibenz(ac)anthracene	$C_{22}H_{14}$	278	DBacA
Benz(a)pyrene	$C_{20}H_{12}$	252	BaP
Dibenz(ah)anthracene	$C_{22}H_{14}$	278	DBahA
Benz(ghi)perylene	$C_{22}H_{12}$	276	BghiP

The three engines had emissions with very different PAH contents. DBahA and DBacA were detected in only a few cases in samples from the swirl chamber engine. The swirl chamber engine's emissions had the greatest PAH-content: the sum of the mutagenic

compounds (except FL and Py) ranged from 32.3 to 1273 ppm for engine A, from 0 to 274.3 for engine B and from 0 to 548.1 ppm for engine C.

The contents of mutagenic compounds, plotted as a function of load (Pe) and number of revolutions (n), are shown in Figures 6 to 8.

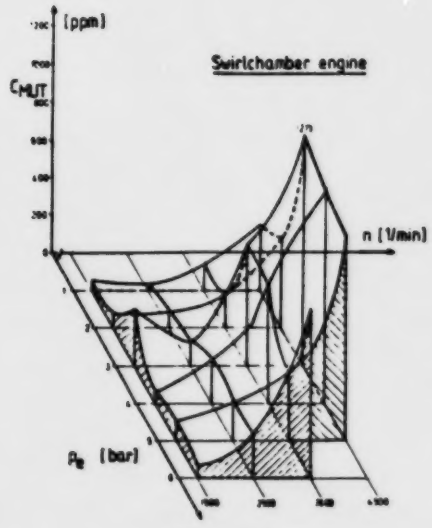


Figure 6

Content of Mutagenic Compounds in Particulate Matter (Swirlchamber Engine)

(Source: Reference 19)

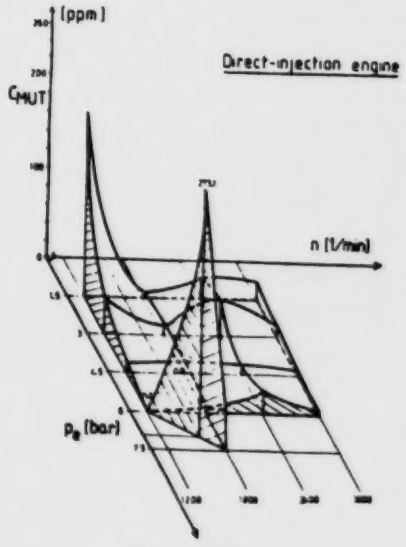


Figure 7

Content of Mutagenic Compounds in Particulate Matter (Direct Injection Engine)

(Source: Reference 19)

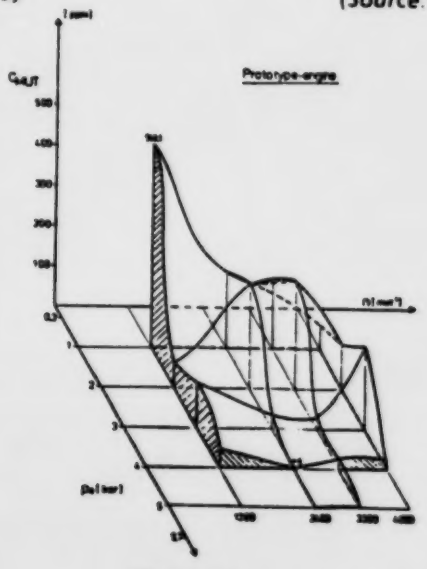


Figure 8

Content of Mutagenic Compounds in Particulate Matter (Prototype Engine)

(Source: Reference 19)

Engine A's PAH emissions increased as the rpm's increased. Engine B gave only two PAH emission peaks, whereas engine C emitted its maximum PAH's at low Pe and n and had significant amounts at other combinations of Pe and n. F1 and Py were the most abundant compounds in soot. More specifically, after F1 and Py, BghiP was present in the greatest quantity followed by BbF and BeP. BaP was detected in the range of 7.8 to 235.8 ppm in emissions from engine A and from non-detectable to 36 ppm for the direct injection engine.

The particle size of emissions was determined with an 8-stage impactor and the size distribution related to PAH content was as given in Table 13.

Table 13

Fractions of the Total Mass of Particulate Matter and of Benz(k)fluoranthene, Benz(a)pyrene and Benz(ghi)pyrene as a Function of Particle Diameter

(Source: Reference 19)

Impactor Stage	*Cut-off Diameter (μm)*	*Collected mass on the Stages*			
		Particulate Matter (%)	*BkF (%)*	*BkF (%)*	*BghiP (%)*
0 - 5	0.9	22.0	n.d.*	n.d.*	n.d.*
6	0.6	7.6	1.2	14.0	4.7
7	0.4	12.8	14.5	14.0	1.4
Backup	0.4	57.4	85.5	72.0	83.8

* n.d.: Not detectable

As can be seen, the PAH's were associated with the smaller particles (diameter <0.4 μm). Moreover, the PAH concentrations were highly correlated among each other and the correlations between PAH particulate concentrations, and/or of the hydrocarbons, would seem to be significant. The correlation analyses suggests the possibility of estimating exhaust's PAH content from the values of the other parameters.

3. EFFECTS OF EXHAUST PURIFIERS ON EMISSIONS

An increasing number of studies report a possible relationship between diesel exhaust exposure and potentially serious toxic effects. With these associations, yesterday's mild objections to the use of diesel engines (eg. the non-esthetic higher noise level, odour and smoke) have been replaced by a more serious concern for health . As a result, polluters have had to re-examine their emission data and, if necessary, decrease emission levels in order to meet more stringent social and regulatory standards. Different types of exhaust purifiers have helped in the latter task.

Hunter et al[17] studied the effect of an oxidation catalyst on diesel exhaust emissions. Measurements were made on a V-8 direct injection, naturally aspirated, 210 hp (at 2800 rpm) Caterpillar diesel engine equipped with an Engelhard PTX platinum exhaust gas purifier. A number of conclusions were drawn based on the data and results. Brake specific NO_X emissions were not affected by the catalysts. There was a brake specific increase in equivalent NO_2 emissions with a similar decrease in NO emissions, at most modes, when the catalyst was used. Figure 9 illustrates these observations. There was a brake specific decrease in hydrocarbons (HC) and in soluble organic fraction (SOF) with the catalyst (Figures 10 and 11).

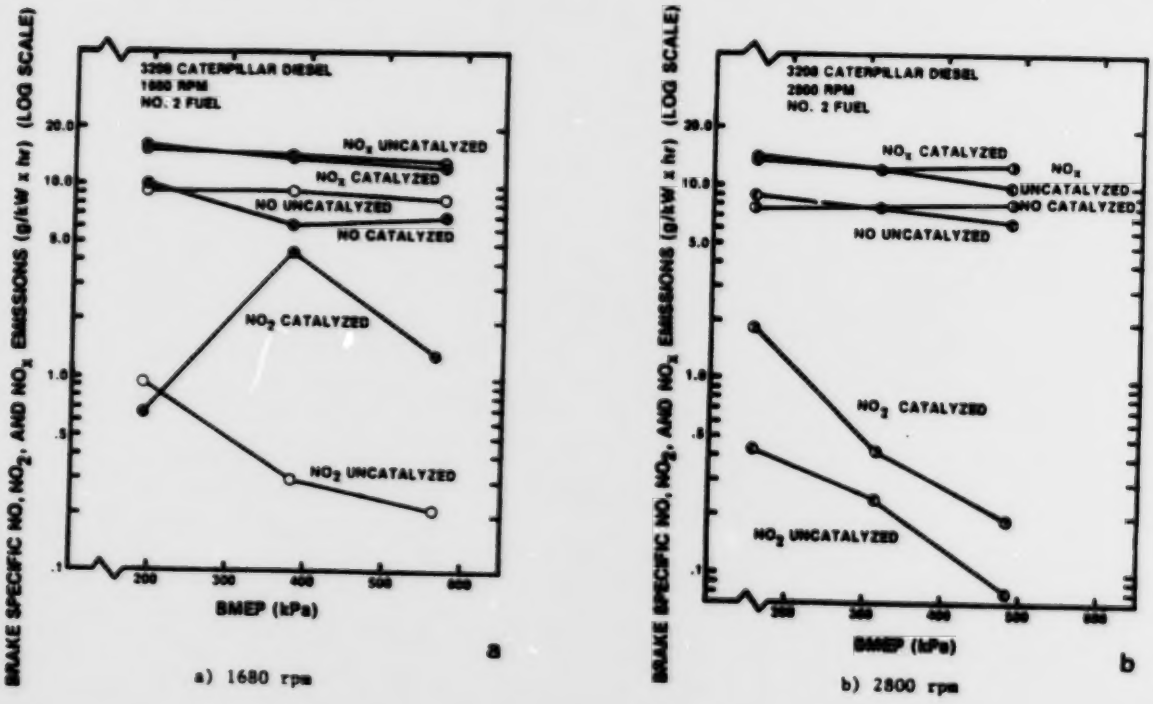


Figure 9

Brake Specific NO, NO_2 and NO_X Emissions With and Without Oxidation Catalyst

(Source: Reference 17)

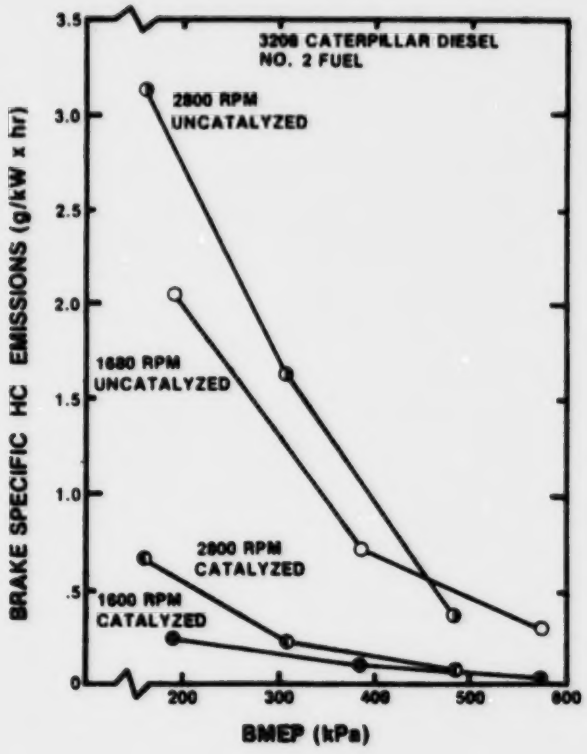


Figure 10

Effect of the Oxidation Catalyst on Brake Specific Hydrocarbon Emissions
(Source: Reference 17)

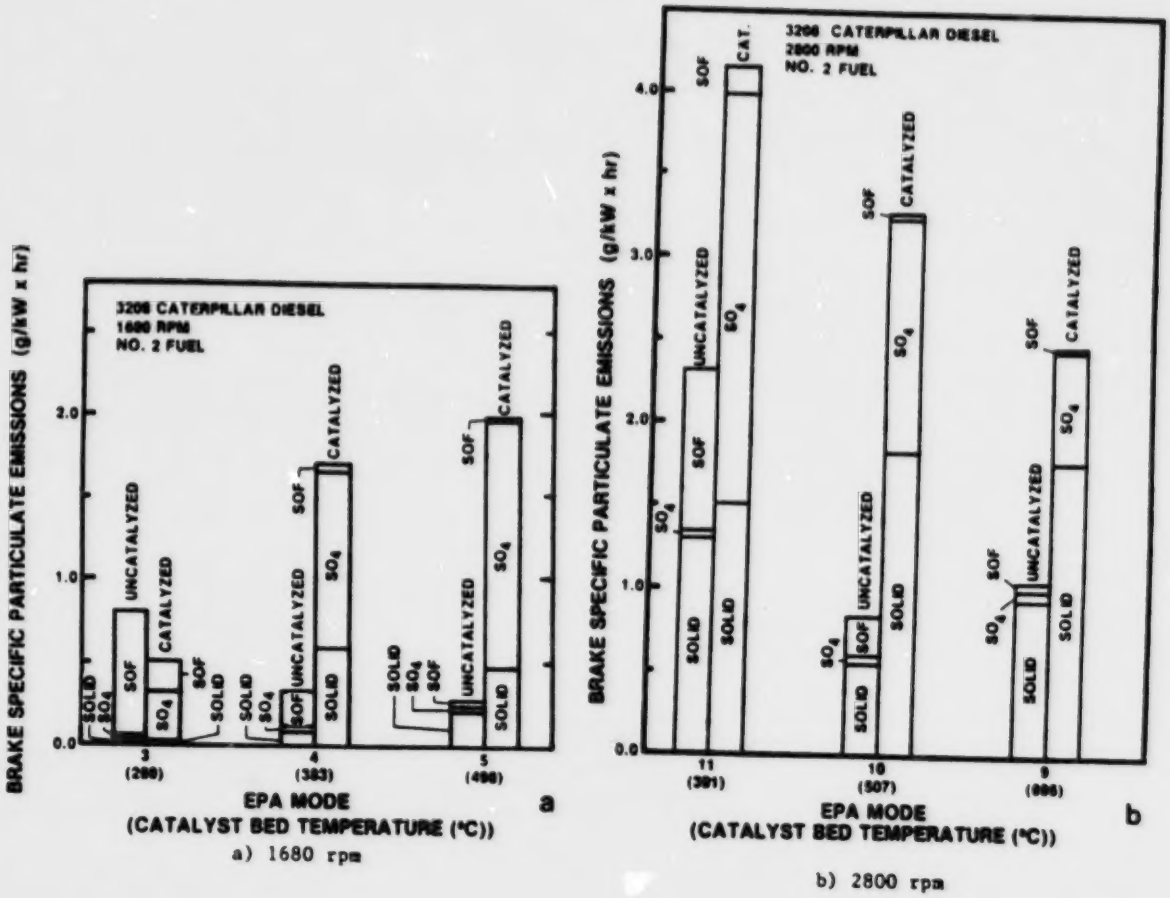


Figure 11

Effect of the Oxidation Catalyst on Brake Specific Particulate Emissions

(Source: Reference 17)

The catalyst caused the oxidation of SO_2 and SO_3 to sulphates, thereby increasing brake specific SO_4 emissions. At certain modes, exhaust opacity and particulate emissions were generally increased by the catalyst. Higher sulphate emissions and a higher portion of unextractable solids from the particulate likely were responsible for these increases.

The percentages of oxygenated, aromatic, and acidic compounds in the SOF were higher. On the other hand, the SOF's paraffinic compounds were decreased by the catalyst.

The mutagenic specific activity of the SOF was generally greater when the catalysts were used. The chemicals were direct acting, since they did not require activation by microsomal enzymes (S-9 fractions), and the mutations they effected were frameshift in nature. The oxygenated acidic and ether insoluble SOF subfractions were also direct-acting, frameshift mutagens (Figures 12 and 13).

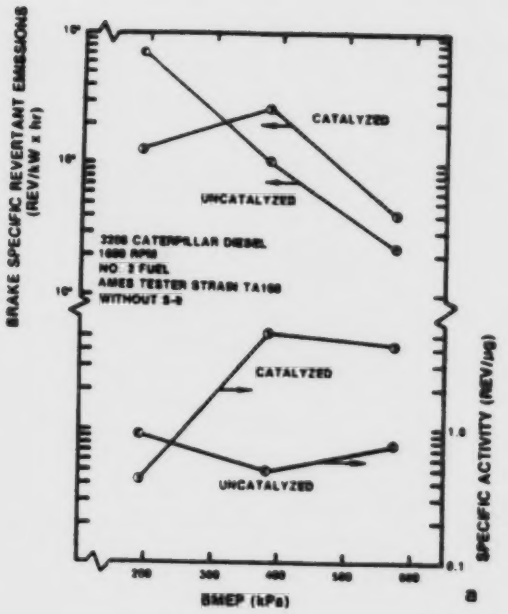
Nonparaffin subfractions had a specific activity that correlated with the NO_2 concentration in the exhaust and diluted exhaust.

Dainty et al[20] have summarized underground mine investigations using ceramic diesel particulate filters and catalytic purifiers. The performance of "unassisted" ceramic diesel particulate filters (DPF's) was assessed. Where the DPF's were "unassisted", neither fuel additives nor catalyst coatings were used to depress the soot ignition temperature. The results showed that the measured efficiency of the filter when fitted to 8- and 10-cylinder Deutz IDI air-cooled engines was low at approximately 40%, compared with the 80 to 90% that was determined subsequently. The low values may have been due to the fact that emissions from other sources were picked up by the measuring equipment. Also measured were CO conversions efficiencies which varied between 30 and 38%, a range of values considered reasonable by the authors. As for respirable combustible dust (RCD) levels, the DPF was estimated to have improved the vehicle operator's environment by 35 to 43% using the Air Quality Index (AQI) criterion.

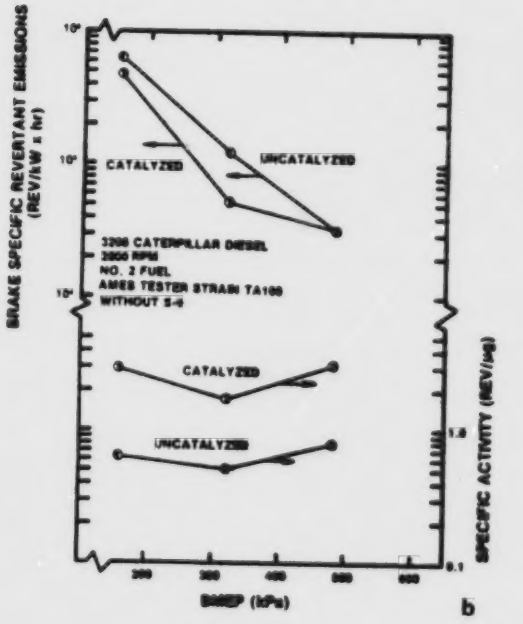
Additive-assisted ceramic DPF's were evaluated. The additives used were 40 mg or 80 mg manganese/L fuel and 20 mg copper/L of fuel. The 80 mg Mn + 20 mg Cu combination appeared to significantly decrease emitted NO_2 concentrations relative to the catalyst. Also, the catalyst CO control efficiency appeared to have been greater than 50%. The filter's efficiency in removing RCD was over 90%.

The use of ceramic DPF reduced the toxicity of the mine environment by 45% for unassisted, filtered exhaust relative to catalytic purifiers in widespread use. Finally, concentrations of gaseous and particulate contaminants, as well as AQI values, generally were well below the maximum acceptable TLV's and AQI value.

The difference between mutagen levels in untreated exhaust emissions or treated with different emission control devices were examined by Mogan et al[21]. The first of two existing purifiers whose performance was reviewed consisted of a bed of alumina pellets impregnated with a platinum oxidation catalyst. Because pelletted purifiers are no longer in general use in Canadian mines (Paul Mogan, personal communication)[82], a review of this devices' performance will not be included.



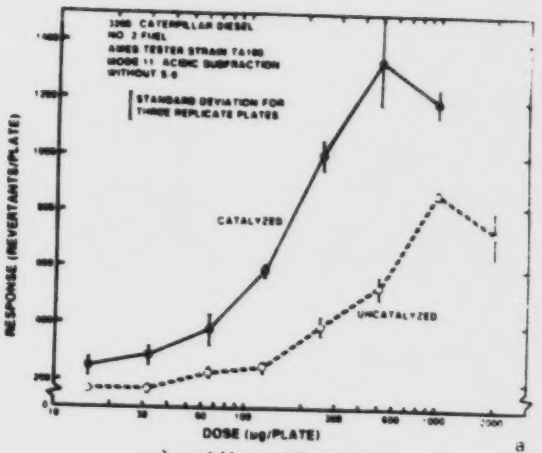
a) 1680 rpm



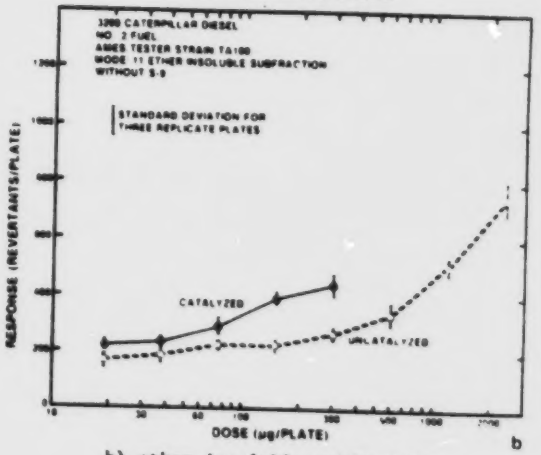
b) 2800 rpm

Figure 12

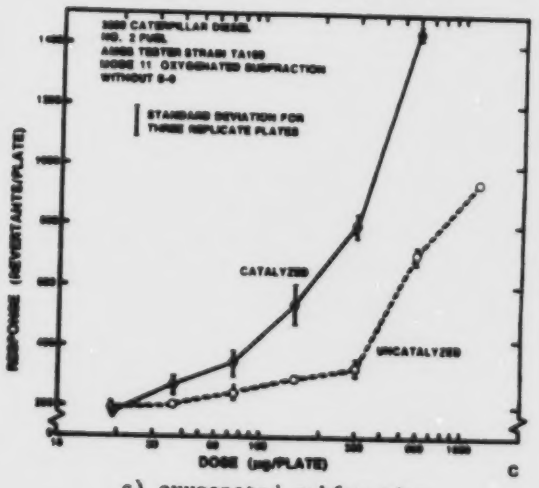
Effect of Catalyst on Brake Specific Revertant Emissions and Specific Activity
(Source: Reference 17)



a) acidic subfraction



b) ether insoluble subfraction



c) oxygenated subfraction

Figure 13

Effect of Catalyst on the Response of Ames Tester Strain TA100 to the Mode 11 Acidic, Ether Insoluble, and Oxygenated Subtractions
(Source: Reference 17)

In monolithic catalytic purifiers, a ceramic honeycomb catalyst support replaced the alumina pellets of the pelletted unit. The former model provided a reduced opportunity for the storage of soot and a smaller increase in the exhaust's mutagenicity confirmed this fact.

The catalytic trap oxidizer, which consists of a catalyst impregnated metal mesh cylinder to trap and subsequently oxidize diesel soot, is the first of two new emission control devices. Mutagen concentrations were reduced to 75% of untreated levels at one operating cycle and, at others, were no higher than 20%. A Corning filter, built similarly to the monolithic catalytic purifier described above except for the plugging of alternate holes in the honeycomb ceramic substrate, was also investigated. The design forces exhaust to travel through the porous ceramic walls, and, consequently creates an efficient diesel soot filter. The trapped diesel soot continuously burns off during high loads. The results from different sources are inconsistent: on one hand, an approximately 90% decrease in mutagenicity was obtained from laboratory trials of steady state, continuous regeneration and additive-induced cycle regeneration; on the other, results from an underground study showed wide swings in mutagenic activity suggesting cyclic storage and release of mutagenic material.

Finally, some data from Hakala et al[14] on the effect on a water scrubber on exhaust gases obtained from diesel engines of unspecified type in a Finnish mine (Table 14).

Table 14

Concentration of PAH's on Exhaust Gases Before and After Water Scrubber

(Source: Reference 14)

	Particulates (mg/m^3)	*Total-PAH (ng/m^3)*	*BaP (ng/m^3)*
Before	15.0	7550	1670
After	5.1	3220	405

The water scrubber reduced the emission of particulates and PAH's about 60 - 80 percent.

4. MUTAGENICITY

Of the compounds known to be adsorbed to diesel exhaust particulate, approximately one hundred have been shown to be mutagenic and several have proven to be carcinogenic in animals(23). The Ames in vitro mutagenicity bioassay is not regarded as a direct measure of cancer risk but it has been endorsed as a means of identifying mutagens and their relative potencies and, also, it is considered a useful indicator of cancer risk(21).

Much of the mutagenic activity in diesel emission soluble organic fractions is likely due to nitro-substituted polycyclic aromatic hydrocarbons (PAH's). For this reason, some of the bacterial strains used are deficient for the nitro reductase enzyme(21). For example, Mogan et al(21) used *S. typhimurium* strains TA98, TA98NR,TA98/1.8- DNP6,TA98NR/1.8-dnp6. Of strain TA98's activity, 50% is due to 1-nitro pyrene and related compounds: 33% stems from supermutagens (dinitro substituted pyrenes); and minor contributions are from non-nitro mutagens. For its part, TA98NR has 10% of TA98's 1-nit opyrene response and it does not activate nitrofurans, nitronaphthalenes, and nitrofluorenes. Consequently, the difference between the response of TA98 and TA98NR (TA98-TA98NR) corresponds to the activity of the latter class of compounds.

TA98/1.8-DNP6 lacks the enzyme that activates supermutagens (1.3-,1.6-, and 1.8-dinitro pyrene). TA98NR- TA98/1.8-DNP6 gives information on the responses of supermutagens. Strain TA98NR/1.8-DNP6 lacks both of the enzymes mentioned above. Therefore, the activity observed with this organism is indicative of the occurrence of non-nitro mutagens(21).

One must be vigilant when interpreting the result of mutagenicity testing because the activity of a sample may be affected by a number of factors. What is more, the reproducibility of results is far from optimal. After a study of interlaboratory variations, Knuiman et al(24) concluded that the substantial variations observed could not be explained.

In the first of the two sections on mutagenicity assessments, the data from bacterial assays are discussed. In the second, the emphasis is on the results obtained from tests performed using mammalian cells.

4.1 MUTAGENICITY RESULTS FROM BACTERIAL CELL SYSTEMS

Most mutagenicity tests are carried out on bacterial cells because the cells are inexpensive, easily obtained and maintained.

Gibbs et al[15] have published data on the mutagenic activity of diesel engine emissions from 20 in-use diesel automobiles subjected to different driving cycles. Table 15 gives average mutagenic activity for the soluble organic fraction (SOF). In general, the FTP cycle gave samples with the highest specific activity.

Table 15

Mutagenic Activity per Microgram of SOF

(Source: Reference 15)

(Revertants/µg SOF)

	FTP	*HFET*	*50 MPH CRUISE*	*IDLE*	*SAMPLE (n)*
GM	3.8	2.4	2.1	2.4	57
VW	11.3	10.9	8.1	3.4	36
MB	4.8	4.4	3.5	2.7	18

Table 16 shows mutagenic response on a particulate mass basis. With some exceptions, the bio-activity on a total particulate basis is uniform within a vehicle type. This is so even though there are large variations in activity among the vehicles tested.

Table 16

Mutagenic Activity per Microgram of Particulate

(Source: Reference 15)

(Revertants/µg Particulate)

	FTP	*HFET*	*50 MPH CRUISE*	*IDLE*	*SAMPLE (n)*
GM	0.77	0.75	0.72	0.43	57
VW	1.95	2.06	1.53	1.94	36
MB	0.53	0.45	0.40	0.39	18

From these findings, it was concluded that " a gram of particulate is a gram of particulate" regardless of driving cycle.

Ong et al[25] conducted a Salmonella/microsome assay to test the mutagenicity of diesel exhaust particulate (DEP) compared to coal dust (CD). The mutagenic activity of urine samples from the exposed rats was also quantitated. CD was not mutagenic with and without microsomal activation. On the other hand, both DEP and DEP plus CD

showed mutagenic activity that was higher in the TA98 than the TA100 strain of *Salmonella typhimurium*, both with and without S9 activation. The number of revertants showed that DEP plus CD extracts were less mutagenic than DEP alone. These results are detailed in Table 17.

Table 17

Mutagenicity Studies of DEP and/or CD Solvent Extracts in *S. typhimurium*

(Source: Reference 25)

Particulate Sample	*Amount of Particulate[a] (mg/plate)*	*His + revertant/plate*			
		TA98		TA100	
		-S9	+ S9	-S9	+ S9
Negative control (DMS)		37	42	100	112
Positive control (2AA)[b]			2114		2450
Clean air	0.0028	31	38	84	116
	0.0056	28	38	88	123
	0.0112	39	38	84	127
	0.0225	39	40	86	138
CD	0.70	35	57	84	131
	1.41	37	61	91	153
	2.83	46	60	95	142
	5.66	46	66	98	145
DEP	0.33	94	141	202	282
	0.65	198	253	415	398
	1.31	191	283	457	488
	2.63	284	445	738	676
CD plus DEP	0.57	66	97	158	217
	1.14	104	137	266	301
	2.28	120	176	300	334
	4.56	182	299	455	446

[a] Concentrations have been converted from the amount of extracted organic material to the amount of particulate

[b] 2.5 µg 2-aminoanthracene/plate

The authors concluded that because DEP was mutagenic and CD was not, that DEP must have been responsible for the mutagenicity of the DEP plus CD extracts. Also, it was deduced that CD and DEP do not act additively or synergistically because the mutagenicity of CD plus DEP was no greater than that of DEP alone[25]. Urine mutagenicity measurements in rats revealed no mutagenic activity after three, six or twenty-four months of exposure. This lead the researchers to postulate four reasons to explain this phenomenon:

1) The concentrations of mutagens in the exposure chambers were too low to be detected by the assay,
2) Very little DEP-associated mutagens or promutagens were released (or dissolved) into the body fluid and excreted through urine,
3) Mutagenic compounds remained in the body, and very little, if any, were excreted, or,
4) Most mutagenic compounds were deactivated before they were excreted.

Morimoto et al[26] have Ames tested the genotoxicity of tar samples from light duty (LD) and heavy duty (HD) diesel engines using TA98 and TA100 strains of *Salmonella typhimurium*. Figure 14 shows that the mutagenic response of both LD and HD tars was positive and dose dependent with TA98 and TA100 strains. Furthermore, metabolic activation systems enhanced the mutagenic effects in strain TA100. Such an increase in response was not observed when enzymatic activation was used with strain TA98.

In Figure 15, the results of tests carried out on neutral and acidic subfractions of LD and HD engine tar samples are illustrated. The neutral subfraction from the LD sample had the highest number of revertants; acidic subfractions from both LD and HD tars were intermediate in their mutagenic activity; and the neutral subfraction from the HD tar sample had the weakest mutagenic response.

Peterson and Chuang[27] obtained data on similar tests using extracts from light duty (LD) and heavy duty (HD) diesel engines. These are reproduced in Table 18.

Again, the LD neutral subfraction had the highest activity, but the HD aromatic subfraction was least mutagenic in this case.

Chan et al[28, 29] have concentrated on the differences in mutagenic activity generated by different sample collection or extraction methods. The following figures were reproduced for two reasons; first they illustrate the fact that variations in assay techniques may affect the results obtained; secondly, and more importantly, they give an approximate range of values in which the mutagenic activity of each fraction of diesel particulate extracts may be found (Figures 16, 17 and 18).

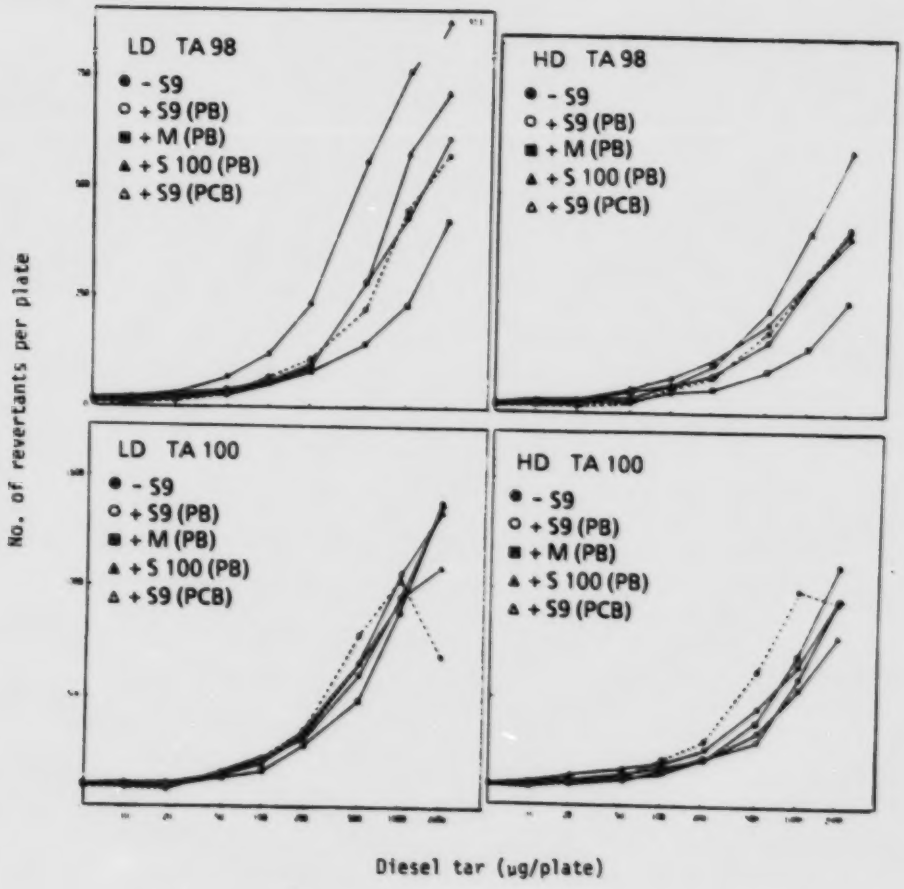


Figure 14

Mutagenicity of LD and HD Tar Samples with Various Metabolic Activation Systems in TA98 and TA100

(Source: Reference 26)

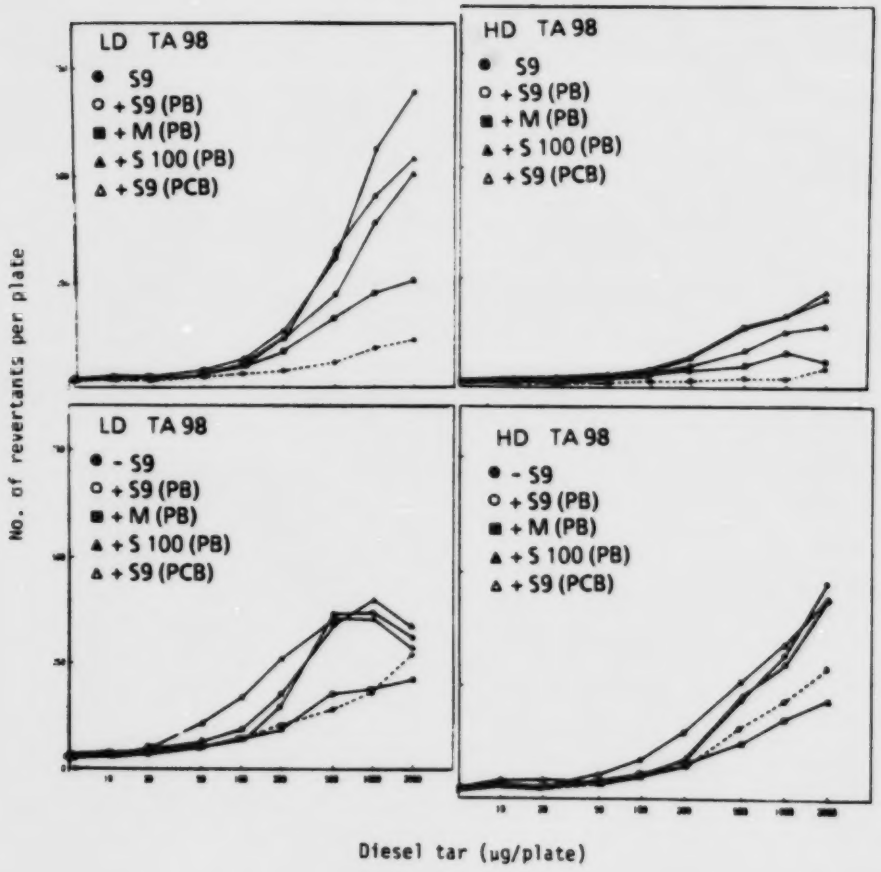


Figure 15

Mutagenicity of Neutral (upper row) and Acidic (lower row) Subfractions of LD and HD Diesel Tar Samples in TA98

(Source: Reference 26)

Table 18

Ames Mutagenesis Bioassay (Total Revertants) of Particulate Extracts and Compound Class Fractions Using Tester Strain TA-98 (Nonactivated)*

(Source: Reference 27)

Engine Type	Total Extract	Base	Acid	Aliphatic	Aromatic	Moderately Polar Neutral	Highly Polar Neutral	Total Fraction Activity (Percent of Total)
LDD I	312,592	2,867	3,593	1,830	20,656	101,434	96,360	226,740 (72)
LDD II	325,180	132	39,855	--	22,227	220,020	25,894	308,128 (95)
HDD	51,824	1,036	14,424	--	777	15,547	18,556	50,340 (97)

(*) Average mutagenic response at 50 µg dose level x mass of extract or fraction

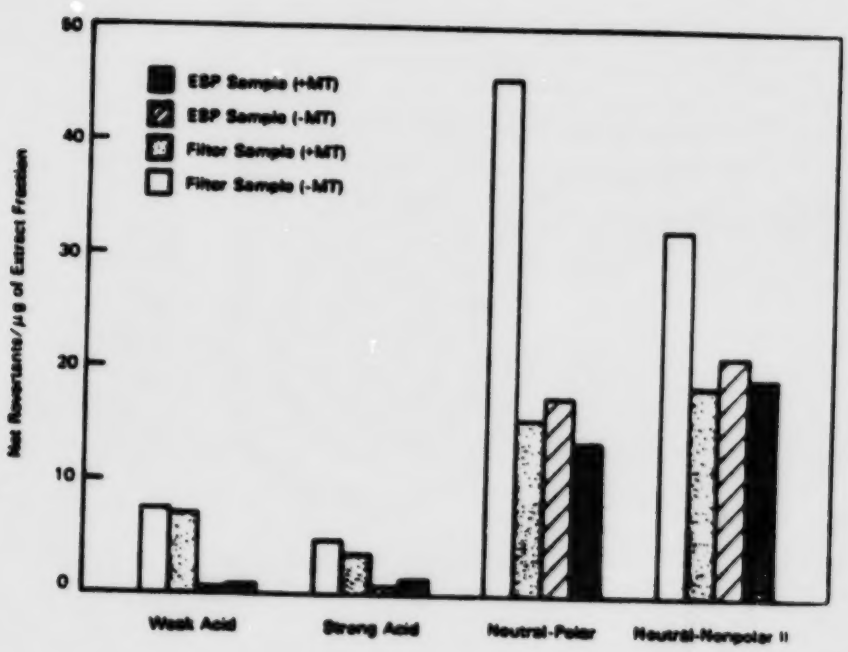


Figure 16

Specific Activity of Diesel Particulate Extract Fractions Determined by the Ames Test (+ MT Indicates Metabolic Activation with S9, -MT Indicates no Activation)

(Source: Reference 28)

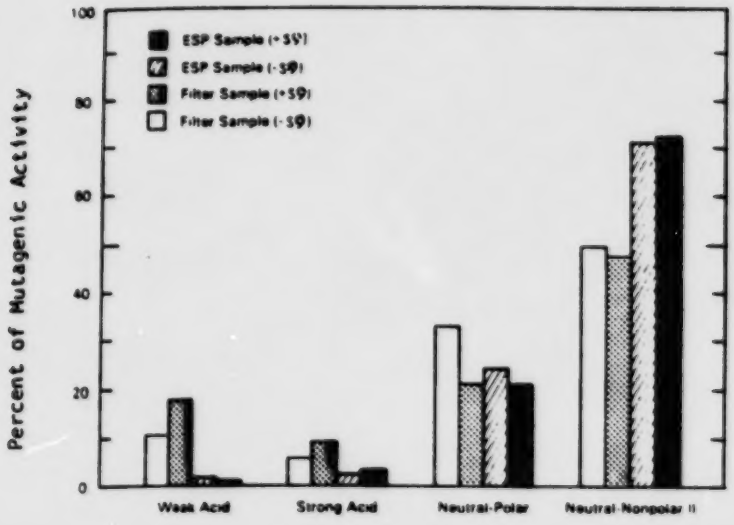


Figure 17

Four Extract Fractions Containing Mutagenic Activity of Diesel Particulate Extracts ESP Indicated Sample was Collected by Electrostatic Precipitation (+ S9 and -S9 Indicated S9 Activation or No Activation in the Ames Test Using TA98)

(Source: Reference 29)

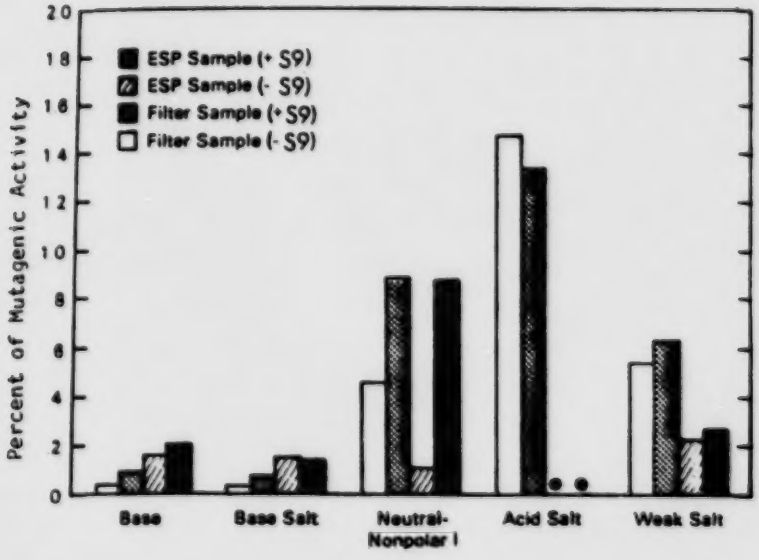


Figure 18

Contribution of Mutagenic Activity in the Less Active Extract Fractions of The Diesel Particulate Extracts (* Below Detection Limit)

(Source: Reference 29)

The review of the results of short-term bioassays by Lewtas and Williams[7] agreed with the data presented when they wrote that polar and highly polar neutral subfractions of compounds that are extractable from exhaust particulate contained most of the mutagenic activity of diesel engine emissions (Table 19).

Table 19

Distribution of the Mass and Bacterial/Mutagenic Activity of Fractional Diesel Particle Organics

(Source: Reference 7)

Fraction	*Mass (%)*	*Specific Mutagenic Activity (rev/mg)*		*Distribution of Mutagenic Activity (%)*	
		-S9	+ S9	-S9	+ S9
Organic acids	14.9	193	248	4.9	9.5
Organic bases	0.3	43.8	132	0.02	0.10
Ether insolubles	3.9	53.9	80.9	0.39	0.80
Paraffins	36.7	Neg	Neg	0.0	0.0
Aromatics	6.9	49.5	30.1	0.60	0.54
Moderately Polar	5.0	7520	2620	64 9	33.5
Highly Polar	26.9	629	798	20.2	55.4
Unfractionated (DCM)	--	2557	1625		

The reviewers quoted studies showing that the fraction containing the most polar and highly oxygenated species was mutagenic as well. Many fluorenones and methylated fluorenones would be among constituents of this group. They also acknowledged the mutagenicity of nitrated-PAH's.

The studies presented below concentrated on the identification of those chemicals most likely to be responsible for the observed mutagenic responses.

Hsieh et al[30] conducted analyses of the genotoxic components in diesel engine emissions. They incubated *Salmonella typhimurium* with 0.0, 14.5 and 37.5 µg of ^{3}H-1-nitropyrene in dimethylsulfoxide. AP-DNA adducts were detected at a level of 3.0 and 4.9 Fmo1/µg DNA (low dose) and 3.7 and 7.0 Fmo1/µg DNA (high dose). Not only do these results support the claim that 1-NP is mutagenic, but also provide evidence for the possibility that the chemical produces tumours by interaction with genetic material.

In gas exposure chambers, Matsushita et al[23] subjected test plates of *Salmonella typhimurium* (strains TA100,TA98 and TA104) and *Escherichia coli* (strain WP2uvrA/pkM101), with and without rat liver microsomal enzymes (S9 fraction) to exhaust from a

4-cycle, 2369 cc, IDI engine that was operated at 1000 rpm and 80% load. It should be noted that this speed/load combination is considered unrealistic and well removed from the engine's maximum design speed (Paul Mogan, personal communication). The exposure periods ranged from 2 to 8 hours. Particulate free exhaust appeared to be mutagenic without S9. Activity decreased in the following order: TA100, WP2uvrA/pkM101 and TA98. TA104 or tests with S9 gave negative results. However, the number of revertants for TA100 and TA104 increased with exposure time. The results showed that gas phase mutagens were direct-acting. When compared to the particle phase of diesel exhaust (particles without the absorbed, extractable fraction), the mutagenic activity of the gas phase was at least 30 times higher. Unfortunately, the researchers did not supply even a tentative list of gas phase compounds.

Because nitro derivatives of benzene are believed to be mutagens in diesel exhaust gas, 94 such compounds were subjected to mutagenicity tests. The results are shown in Table 20.

As can be seen, among the substances tested, 33 were not mutagenic and 61 were mutagenic to TA100 and/or TA98 strains.

Similarly, 50 polycyclic aromatic hydrocarbons (PAH's) from diesel exhaust were tested for mutagenicity using TA100 and TA98 strains. As listed in Table 21, twenty-two were non-mutagens and 28 were mutagens.

Some dinitropyrenes (DNP's) were also tested. The activity, in decreasing order of magnitude, was as follows: 1,8-, 1,6-, 1,3-, 1,7-, 2,7-, 4,10-, 4,9- and 4,5- DNP. All of these and 2- and 4- nitropyrene (NP) had higher activity than 1-NP.

Nitrated pyrenes appear to be direct acting (i.e. they do not require bioactivation by S9). In tests, the direct-acting mutagenic activity of 1,6-DNP decreased, whereas the promutagenic activity increased with the addition of diesel extracts, PAH's, or neutral compounds..

In a related mutagenicity study, Tokiwa et al[31] tested different nitro-fluoranthenes (NF) and nitropyrene (NP) derivatives in a number of Salmonella typhimurium tester strains (Table 22).

The nitro derivatives induced frameshift type mutations. DNP's and DNF's were strongly mutagenic for all *S. typhimurium* strains except TA1535. Dinitro derivatives were the most potent derivatives of the nitroarenes.

Table 20

Mutagenicity of Nitro Derivatives of Benzene for *Salmonella Typhimurium* TA100 and TA98 Strain

(Source: Reference 23)

Non-Mutagens (33 Species)

Nitrobenzene
p-Chloronitrobenzene
p-Ethylnitrobenzene
2,4-Dinitrophenol
o-Nitroaniline
m-Nitrotoluene
3-Nitro-o-xylene
4-Nitro-m-xylene
2-Nitro-p-toluidine
o-Nitrobenzene sulfonic acid
2,4-Dinitrobenzene sulfonic acid
o-Chloronitrobenzene
Pentachloronitrobenzene
o-Nitrophenol
2,6-Dinitrophenol
p-Nitroaniline
p-Nitrotoluene
4-Nitro-o-xylene
5-Nitro-m-xylene
m-Nitroanisole
m-Nitrobenzene sulfonic acid
o-Nitrobenzaldehyde
m-Chloronitrobenzene
o-Ethylnitrobenzene
p-Nitrophenol
2,4,6-Trinitrophenol
o-Nitrotoluene
4-Chloro-2-nitrotoluene
2-Nitro-m-xylene
3,5-Dinitro-o-xylene
2,4-Dinitrobenzoic acid
p-Nitrobenzene sulfonic acid
3,5-Dinitro-o-cresole

Mutagens (Less than 10^3 revertants/mg: 26 Species)*

o-Dinitrobenzene (TA100,-S9)
3,4-Dinitrophenol (TA100, + S9)
2,4,6-Trinitrophenol (TA100, + S9)
2,6-Dichloro4-nitroaniline (TA98, -S9)
m-Bromonitrobenzene (TA100, + S9)
2,3-Dinitrotoluene (TA100, + S9)
2,6-Dinitrotoluene (TA100, -S9)
2,4-Dinitro-m-xylene (TA100, -S9)
o-Nitroanisole (TA100, -S9)
m-Nitrobenzoic acid (TA100, -S9)
2,5-Dinitrobenzoic acid (TA98, -S9)
m-Nitrobenzaldehyde (TA100, + S9)
o-Nitrophenylhydrazine HCl (TA100, -S9)
m-Nitrophenol (TA100, + S9)
2,4,6-Trinitrophenol (TA100, + S9)
2-Chloro-4-nitroaniline (TA98, + S9)
2-Methyl-4-nitroaniline (TA98, -S9)
p-Bromonitrobenzene (TA100, + S9)
2,4-Dinitrotoluene (TA100, -S9)
3,4-Dinitrotoluene (TA100, -S9)
3-Nitro-p-toluidine (TA100, -S9)
o-Nitrobenzoic acid (TA100, -S9)
p-Nitrobenzoic acid (TA100, -S9)
3,4-Dinitrobenzoic acid (TA100, -S9)
p-Nitrobenzaldehyde (TA100, + S9)
Parathion (TA100, -S9)

Mutagens (10^3 - 10^4 revertants/mg: 22 Species)*

m-Dinitrobenzene (TA98, S-9)
2,4-Dichloronitrobenzene (TA100, + S9)
3,4-Dichloronitrobenzene (TA100, -S9)
2,5-Dinitrophenol (TA98, -S9)
2,4-Dinitroaniline (TA98, + S9)
6-Chloro-2,4-dinitroaniline (TA100, -S9)
2-Nitro-p-xylene (TA100, + S9)
3-Nitro-4-chlorobenzoic acid (TA100, -S9)
o-Nitrobenzylchloride (TA100, -S9)
o-Nitrobenzoyl chloride (TA100, + S9)
p-Nitrobenzoyl chloride (TA100, + S9)
p-Dinitrobenzene (TA100, -S9)
2,5-Dichloronitrobenzene (TA100, -S9)
2,4,6-Trinitrochlorobenzene (TA100, -S9)
m-Nitroaniline (TA98, + S9)
2-Nitro-4-chloroaniline (TA98, + S9)
o-Bromonitrobenzene (TA100, + S9)
p-Nitroanisole (TA100, -S9)
3,5-Dinitrobenzoic acid (TA100, + S9)
m-Nitrobenzylchloride (TA100, -S9)
m-Nitrobenzoyl chloride (TA100, -S9)
3,5-Dinitrobenzoyl chloride (TA100, -S9)

Mutagens (More than 10^4 revertants/mg: 13 Species)*

1,3,5-Trinobenzene (TA100, -S9)
2,3-Dinitrophenol (TA100, -S9)
3,5-Dinitroaniline (TA100, -S9)
2,4-Dinitrofluorobenzene (TA100, -S9)
3,5-Dinitrobenzylchloride (TA100, -S9)
2,4-Dinitrophenylhydrazine (TA98, -S9)
Nitramine (TA100, -S9)
2,4-Dinitrochlorobenzene (TA100, -S9)
2,6-Dinitroaniline (TA98, -S9)
2-Bromo-4,6-dinitroaniline (TA98, + S9)
p-Nitrobenzylchloride (TA100, -S9)
p-Nitrophenylhydrazine (TA100, -S9)
2,4-Dinitrophenylthiocyanate (TA100, -S9)

(*) Symbol in parentheses indicates test conditions in which highest mutagenic activity was obtained

Table 21

Mutagenicity of Polycyclic Aromatic Hydrocarbons (TA100, + S9)

(Source: Reference 23)

Non-Mutagen (22 PAH)

Naphthalene*
2,3,6-Trimethylnaphthalene
Acenaphthylene
3-Acetylphenanthrene
1-Methylfluorene
9,10-Diphenylanthracene
Rubrene
Di-ideno(1,2,3-cd:1',2',3'-1m)perylene
2,3-Dimethylnaphthalene
Diphenyl
Anthracene*
Fluorene*
p-Terphenyl
Pentacene
Pyranthrene
2,6-Dimethylnaphthalene
Acenaphthene*
Phenanthrene*
3-ACetylfluorene
Dibenzo(c,g)phenanthrene
Dibenzo(a,1)pentacene
Ovalene

Mutagens (28 PAH)

2-phenylnaphthalene
9-Methylanthracene#
Triphenylene#
1-Methylpyrene*#
7,12-Dimethylbenz(a)anthracene#
Diben(a,c)anthracene#
Perylene*
Benzo(k)fluoranthene*
Indeno(1,2,3-cd)pyrene*#
Benzo(ghi)perylene*#
2-Methylfluorene#
9-Methylphenanthrene
Fluoranthene*#
Chrysene*
3-Methylcholanthrene#
Dibenz(a,h)anthracene#
Benzo(b)fluoranthene*
Benzo(e)pyrene*
Dibenzo(a,e)pyrene*#
2-Methylanthracene*
Tetracene
Pyrene*
Benz(a)anthracene*#
Benzo(a)pyrene*#
Picene*
Benzo(j)fluoranthene#
Benzo(b)chrysene
Dibenzo(a,h)pyrene*#

---

* PAH detected from diesel exhaust: # PAH showed positive response to Salmonella typhimurium TA 98 in the presence of S9 mix: Carginogen; Cocarcinogen

Table 22

Mutagenicity of Nitrofluoranthenes (DNF's) for Salmonella Tester Strains

(Source: Reference 31)

Compound	*Revertants per µg in S. typhimurium His⁻ strains*						
	TA98	*TA100*	*TA1538*	*TA1537*	*TA1535*	*TA98NR*	*TA98/1,8-DNP$_6$*
3-NF	19,700	9,660	3,280	194	0	6,115	1,365
3,7DNF	422,000	108,000	43,370	16,800	0	122,000	144,000
3,9-DNF	355,000	72,330	105,700	10,800	0	103,000	85,300
3,4-DNF	15,500	11,020	1,550	90	0	5,190	3,040
1-NP	1,901	1,465	704	246	0	773	311
1,3-DNP	293,705	102,798	38,400	10,397	0	72,959	6,220
1,6-DNP	431,064	41,597	43,200	31,598	0	201,998	18,000
1,8-DNP	909,908	189,600	99,600	70,400	0	1,048,000	48,000

The same researchers used the rec-assay with *Bacillus subtilis* to identify chemical mutagens which induce cellular mutations causing DNA-damage. All the NP^- and NF^- derivatives tested inhibited the growth of strain M45 (Rec) much more than H17 (Rec^+) and yielded positive results on the rec-assay system. In particular, they markedly inhibited the growth of strain M45 (Rec^-) at very low doses. The results suggest that the compounds induce DNA damage in this system (Table 23).

Table 23

Results of *Bacillus subtilis* Rec-assay for Nitropyrene and Nitrofluoranthenes
(Source: Reference 31)

Compound	*Dose (µg)*	*Inhibition zone (mm) in B. subtilis*	
		H17 (Rec+)	*M45 (Rec-)*
Dimethyl sulfoxide		0.0	0.0
1-NP	1.0	0.0	5.5
1,3-DNP	0.1	1.0	3.0
1,6-DNP	0.04	1.3	4.5
1,8-DNP	0.02	0.8	2.6
3-NF	0.4	1.3	18.4
3,7-DNF	0.02	0.0	5.7
3,9-DNF	0.02	0.0	4.5

4.2 MUTAGENICITY RESULTS FROM MAMMALIAN CELL SYSTEMS

In the 1984 report, the results of a study by Peirano[1, p. 453] was cited as evidence for the unavailability of compounds from exhaust particulates. Coupled with this observation was the assumption that the risk from these chemicals was less than previously expected. Lewtas and Williams[7] presented evidence opposing the findings mentioned above. They quoted King et al[32, 33] who concluded these compounds could be removed from soot particles after incubation with either serum or lung cytosol. The only hamper on bioavailability according to these particles, would be the binding of the mutagens to serum or lung cytosol proteins or, alternatively, their metabolism to non-mutagenic metabolites by macrophages.

Ong et al[25] performed two different tests on mammalian cells. First considered was a micro nucleus assay on bone marrow cells from the femur of six month exposed mice. The second was a sister chromatid exchange (SCE) assay on blood from rats exposed for three and twenty-four months. Experimental conditions included exposure to diesel exhaust particulate (DEP) coal dust (CD), or DEP plus CD[25].

The frequency of micronuclei in polychromatic erythrocytes from mice exposed to any of the emissions was not significantly different from those observed in controls. Similarly, SCE assay did not reveal any treatment related differences between the exposed group and controls. It was concluded that the exposure of animals to DEP or the combination of DEP and CD by inhalation does not cause genetic changes detectable using the SCE and chromosome aberration assay systems.

Kawabata et al[34] performed unscheduled DNA synthesis (UDS) tests on *in vitro* exposed tracheal rings from rats. They wanted to determine the importance of diesel particulate on DNA damage and repair in the cell nucleus of tracheal epithelia and on lung tumourigenesis. The results of UDS tests on rat tracheal cells incubated with suspensions of diesel exhaust particulate at different concentrations and ^{3}H-thymidine are tabulated in Table 24. These data demonstrated a higher percentage of UDS cells and a significant increase in grain number per UDS cell with increasing concentration of diesel exhaust suspension.

Table 24

Percentage and Grain Numbers of UDS Cells According to Concentration of Added Diesel Particulate

(Source: Reference 34)

	UDS Cell Percent	*Grain Numbers per UDS Cell*
Control	23.43	1.24
Particle 0.125 mg	20.28	1.36
0.25 mg	24.57	1.35
0.5 mg	25.14	1.34
1.0 mg	31.14	1.54*
2.0 mg	34.29	2.68**

(*) $P<0.05$ (**) $P<0.01$

In culture systems from hamster and human epithelial tissue and mixed tissue of the respiratory tract, Mohr et al(35) attempted to assess the biological activity of environmental carcinogens. Different fractions of exhaust condensate from a motor vehicle, whose engine type (i.e. gasoline or diesel) was not specified, were tested for transforming effect on cultures of hamster lung cells. Fractions containing total exhaust, methanol (70%) and hexane, and PAH's exhibited slight growth stimulation at the same degree of dilution. Benzo(a)pyrene did not transform cells of the M3E3/C3 line on its own, but did when insulin, hydrocortisone, estradiol and EGF were added. Diethylnitrosamine decreased the number of surviving colonies with increasing dose. Finally, benz(a)anthracene, benz(a)acridine, benzo(b)fluoranthrene and benzo(k)fluoranthene induced histopathological changes such as metaplasia, dysplasia, or exophytic growth.

Hsieh et al(30) also conducted similar tests on mammalian cells. Mouse embryo fibroblast 10T1/2 cells were treated with 1-nitropyrene (1-NP), and 1,3-, 1,6-, 1,8-DNP or benzo(a)pyrene BaP (positive control) at 1 µg/mL or with diesel particulate extract (DPE) up to 10 µg/mL for 24 hours. The aerobic treatment of 10T1/2 cells with 1-NP failed to lead to DNA adducts at the sensitivity level available (approximately 5 adducts per 10^6 bases). However, DNA adducts were consistently detected in cells exposed to 1,8-DNP (approximately 5 adducts/10^6 bases) and very weakly in some samples of 1,6- and 1,3-DNP treated cells.

The group also exposed male and female F344 rats to exhaust generated by an Oldsmobile 5.7 L engine operated according to Federal Test Procedures urban driving cycle. Exhaust, diluted to 7.1 mg/m^3 was introduced into the exposure chamber 7 hours/day, 5 days/week. Control chambers received clean, filtered air only. Using an extraction procedure to enhance the sensitivity of the assay, they were able to detect DNA adducts in some of the samples.

In a related study, the researchers attempted to detect AP-DNA adducts in animal livers: rats were treated subcutaneously in the suprascapular region on the day of birth and then weekly for an additional seven times with 100 µc 1-NP per kilogram body (70 mMol DMSO). Twenty-four hours after the last injection the animals were sacrificed, and liver DNA was isolated and the AP-DNA adducts quantitated. AP-DNA adducts were detected in the liver DNA of the two treated female rats, but not in treated male rats or in control animals.

5. DEPOSITION AND CLEARANCE OF INHALED SUBSTANCES

The 1984 publication of this report included evidence for the differential deposit of exhaust particulate in various regions of the respiratory tract. Also, there was support for a model of anatomy according to which the clearance of deposited particles would be governed by a two phase mechanism, depending on the area of deposition: from the compartment consisting of the respiratory bronchioles and all the structures connected with the alveolar clearance would proceed more quickly because it involves the relatively rapid mucociliary escalator; the second clearance phase takes place from pulmonary alveoli because clearance depends on macrophages coupled to the mucociliary transport process in this compartment. This process is more time-consuming.

A presentation of the deposition and clearance of inhaled substances is discussed below. But, before getting into this material, it may be helpful to review the pattern of particle deposition in the respiratory tract, and at the same time, the basic physiology of this elaborate system for gas exchange.

Particles with a diameter less than 5 µm are considered respirable. Because 95% of particles are said to be less than 1 µm in diameter, diesel exhaust particulate is largely respirable. The first line of defense of the respiratory tract is an aerodynamic one. Usually, particles larger than 2 or 3 µm in diameter impact on the nasal passages and are retained in the hair, cilia and mucociliary escalator of the irregularly structured nasopharyngeal cavity. In the tracheobronchial tree aerodynamics are still important. A combination of turbulent air movement, with the presence of the mucociliary escalator, ensures the removal of more particles in a process that may last a few hours or longer. Particles 0.05 µm to 2 µm deposit by gravitational sedimentation at the bronchial-bronchiolar level in the lungs. Finally, those ranging in size from 0.01 µm to 0.05 µm are particularly likely to settle in the alveoli and are removed by macrophages. These cells are derived from monocytes. They engulf or phagocytose potentially harmful particulate matter in the alveoli and eventually reach the mucociliary escalator to carry these out of the respiratory system. These processes can be completed within a few weeks or may require as many as a few years. During this time, the particles deposited remain in intimate contact with approximately 30 m^2 of microscopically-thin alveolar membranes which separates the blood stream from the inhaled air.

The extent of exhaust particle deposition and clearance at the alveolar level may ultimately affect the accumulation and biological risk that exposure to particulate matter represents. Because of the possible detrimental effects of particulate

accumulation, researchers have investigated the extent and rate of particulate clearance from the lungs of experimental animals.

The results of the first few articles in this section either review information that was known at the time of the 1984 report or they present new information to support it. The remaining reports introduce the research done on new topics, including the effects of particle nature on accumulation, including an examination of macrophage activity.

In 1983, Griffis et al(36) completed a study that dealt with the clearance of soot particles from rat lungs after subchronic diesel exposure. The diesel exhaust emissions were generated by an Oldsmobile 5.7 L diesel engine operated on a 7 mode urban cycle. Fisher 344 rats were exposed to three concentrations of diesel soot (4100, 940 and 150 µg/m3) (values corrected to take into account the particulate concentration in control chambers)for 7 hours/day, 5 days/week, for a total of 541 hours over an 18-week period. To determine clearance of soot particles after exposure, male and female rats from each exposure levels were killed and their lungs examined at 1 day and 1, 5, 15, 33, and 52 weeks after the termination of exposure. One day after the subchronic exposure period ended, the lung burden values were those found in Table 25.

Table 25

Lung Burdens of Diesel Exhaust Soot in Rats One Day After and 18-Week Exposure to Diluted Exhaust

(Source: Reference 36)

Exposure Group	*Average Net Aerosol Conc. (µg/m3)*[a]	*Predicted Lung Burden*[b] *(µg/g lung)*	*Net Lung Burden of Soot µg/g lung ± S.D., n = 8*	*Lung Burden Normalized for Exposure Concentration*[c] *[(µg/g lung)/ (µg/m3)]*
Control	0	0		
Low	150	47	35 ± 8	0.23
Medium	940	290	220 ± 38	0.23
High	4100	1280	1890 ± 330	0.46

a Particulate levels found in the control chambers have been subtracted from total particulate levels
b Estimated from deposition of ^{67}Ga-labeled diesel particles in rats (Wolf et al, 1981b)
c Net lung burden divided by average aerosol concentration

The results show that high exposure to diesel exhaust particles (4100 µg/m3) produced disproportionately high lung burdens of particles. This observation is supported by the

fact that the lung burdens were twice as high for the high level exposure group as they were for the other two. To explain these results the authors suggested that the excess lung burden at the high exposure level was due to impairment in clearance, since clearance half-time was double for this group compared to that of the other two. Half times were as follows: low: 87 ± 28 days; medium: 99 ± 4 days; high: 165 ± 8 days. They also suggested that this doubling of clearance half-time in a subchronic study implies impairment early on in the exposure period.

Figure 19 illustrates that there was an apparent lack of a rapid, early clearance phase. In fact, the opacity of the accumulated soot in the lungs acted as a dark background through which the early clearance phase could not be seen.

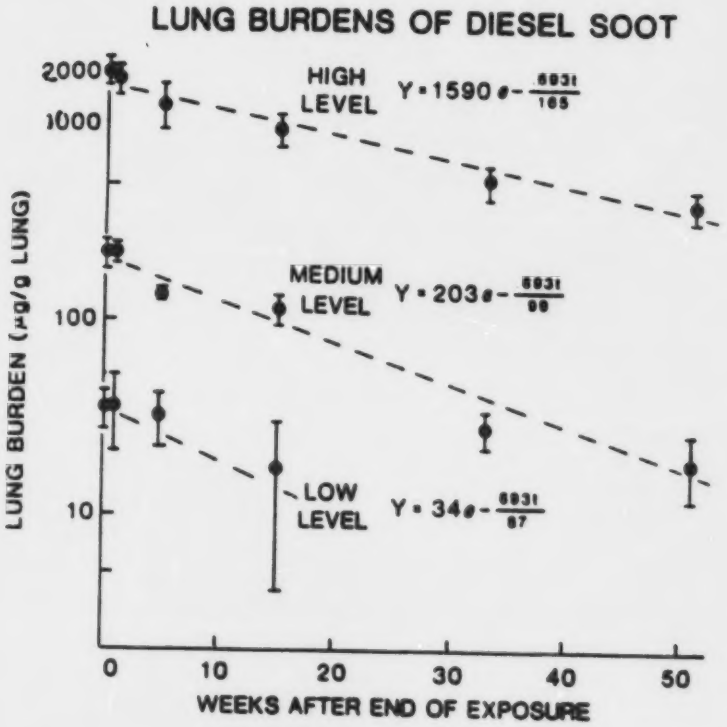


Figure 19

Lung Burdens (mean ± SD; n = 8) in Rats at Various Times Following Cessation of 18 Weeks Diesel Exhaust Exposure at High, Medium and Low Exposure Levels (Top to Bottom, respectively)
(Source: Reference 36)

Particle accumulation and clearance in rat lungs were studied by Wolff et al[37] as well. In this case, rats were subjected to three soot concentrations (0.35, 3.5 and 7.0 mg/m^3) 7 hours/day, 5 days/week for 30 months. Diesel exhaust was generated by a 1980, 5.7 L Oldsmobile V-8 diesel engine using D-2 diesel fuel. The exposure atmospheres are described in Table 26.

Table 26

Exposure Atmospheres Through 30 Months of Exposure[a]
(Source: Reference 37)

	High		Medium		Low		Control	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Particles (µg/m^3)	7080	810	3470	447	353	71	13	6
Carbon dioxide (ppm)	6640	1320	4360	590	2280	371	2010	390
Carbon monoxide (ppm)	29.7	12.9	16.5	7.1	2.9	1.0	1.0	0.7
Hydrocarbons (ppm)	13.4[b]	8.3	8.7	5.2	3.8	0.9	2.6	0.6
Nitrogen dioxide (ppm)	0.7	0.5	0.3	0.2	0.1	0.1	0	0
Nitrogen oxide (ppm)	10.0	2.6	5.7	1.5	0.7	0.3	0	0
Ammonia (ppm)	0.7	0.6	0.9	0.9	1.4	1.3	1.1	3.0

[a] Data for particles are from start of study through 30 months. Data for gases are from the 35th week through 30 months.
[b] Methane equivalents

It was found that deposition of emitted particles did not change significantly following diesel exhaust exposure. Tracheal mucociliary clearance in exhaust exposed and in clean air were not significantly different from the 6th to the 30th month of exposure, regardless of the soot level. However, pulmonary clearance was slower in the medium and high level groups. More specifically, it was found that clearance impairments were statistically significant by 6 months for groups exposed to high soot concentrations and by 18 months for those subjected to medium particulate levels. The clearance half-times were 79 ± 5, 8 ± 5, 264 ± 50, 240 ± 50 days for the control, low, medium and high exposure groups, respectively. Associated with these findings was the observation that lung burdens increased progressively at the medium and high exposure levels. In contrast, lung burdens for the lowest level were much lower and only increased late in the exposure. Figure 20 plots the clearance patterns.

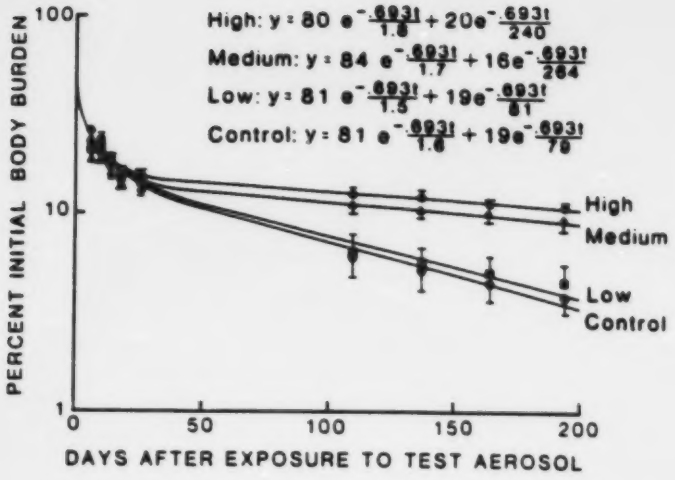


Figure 20

Long-Term Clearance in Rats of ^{134}Cs-FAP Particles Inhaled After 24 Months of Diesel Exhaust Exposure at Concentrations of 0 mg/m³ (●), 0.35 mg/m³ (■), 3.5 mg/m³ (●) and 7.0 mg/m³ (▲). Points on curves are means ± SE

(Source: Reference 37)

Based on these findings, the authors concluded that particulate accumulation occurred non-linearly between the lowest and the two highest exposure levels. They also suggested that increases in lung burdens probably were due to impaired lung clearance mechanisms and not to changes in particle deposition since it was found that deposition remained constant throughout the exposure period.

The same researchers also attempted to determine whether lung burden data were consistent with the data from radiolabelled particle clearance measurements. To do so, they simulated deposition and clearance rates and, also, the amounts accumulated in the lungs. The simulations showed that a decrease in pulmonary clearance had the greatest impact on accumulation results, as expected from earlier observations. More specifically, if pulmonary clearance remained normal for 9 months, for high and medium exposure levels, and if there was a rapid linear transition by twelve months to nonclearance of particles, the predictions provided a reasonable approximation of the observed data (Figure 21).

The data provided evidence for changes that would impair clearance and involve some increase in sequestering of particulate early in the exposure period. However, later there are many areas where particles and macrophages seem to become trapped in fibrous connective tissue. This observation is suggestive of substantial sequestration.

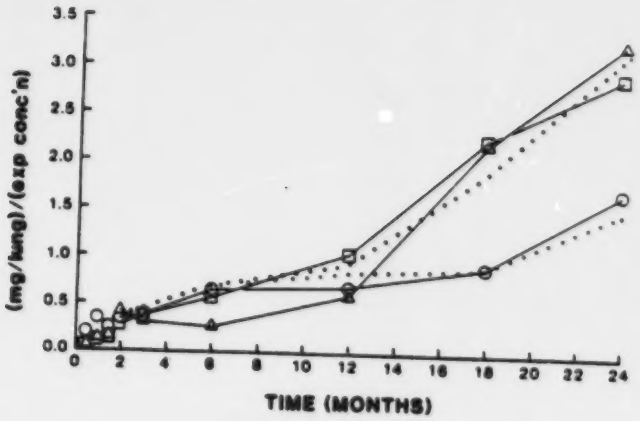


Figure 21

Accumulated lung burdens of diesel soot with time were normalized by dividing the exposure concentration. Data are shown as the means for the exposures of 0.35 mg/m³ (○), 3.5 mg/m³ (△), and 7.0 mg/m³ (□). Simulations are shown of accumulated lung burdens for the medium and high level exposures, assuming normal clearance of diesel soot until 9 months and then a transition to complete sequestration by 12 months for both exposure concentration (···). The simulation for the low level exposure assumed normal clearance until 18 months and then a transition to complete sequestration by 21 months and is shown by the lower dotted line.

(Source: Reference 37)

Wolff et al[38], in the summary figure (Figure 22) presented below, obtained similar results. Lung accumulations for the lowest levels of exposure (0.35 mg/m³) are 2-3 fold less than those from the higher exposures (3.5 and 70 mg/m³)at times past 12 months.

The data suggest impaired clearance at high exposure levels. Since deposition did not change significantly during the exposure period it was not considered responsible for soot accumulation.

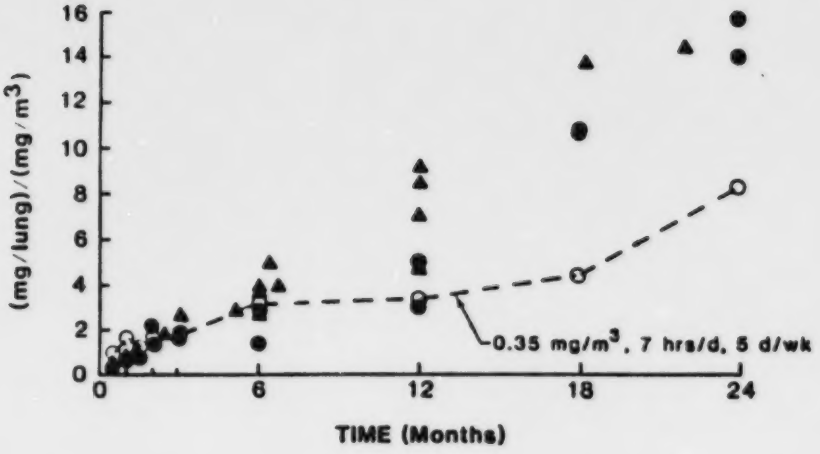


Figure 22

Lung Burdens of Diesel Soot (mg in lung) Divided by Average Daily Concentration [mg/m³ x (exposure hours/day) ÷ 24 x (exposure days/week ÷ 7].
Data are from Strom et al[27] (▲) and Wolff et al[15] (●, o)
(Source: Reference 38)

The results presented thus far indicate that there is a significant increase in the lung to body mass ratio and in clearance half-times with particulate concentrations of at least 3.5 mg/m³. Comparable results were detailed in references 46, 51, 57 and 63 for lung burdens and in references 44 and 60 for clearance half times.

Kawabata et al[34] reported on clearance of diesel particles and epithelial proliferative changes in the lungs of rats after intra-tracheal instillation of a 5 mg/mL suspension of diesel particles. A suspension (0.2 mL) was instilled once a week for 1, 4 or 10 weeks. As presented in Figure 23 the instilled particles accumulated in the lungs and increased lung dry weight in a dose related manner. The data also shows that the instilled particles were retained in the lungs for a long period of time, which is indicative of an incomplete clearance mechanism, even without inhalation of toxic gases.

In the 10 mg group epithelial proliferation was observed in a limited area (0.1%) six months after the instillation of diesel particles. It increased to 1.5% by 18 months, but was not measured at 24 months because of many tumour formations. Proliferation was observed but was practically insignificant in the 4 mg group at 18 months. There was no sign of it in the control group.

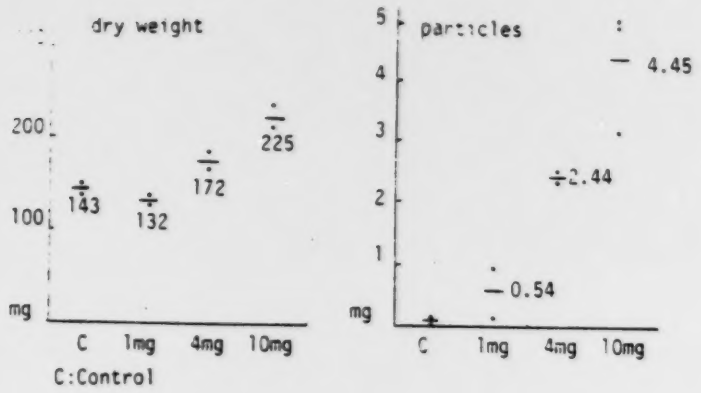


Figure 23

Dry Weight of the Lungs and Remaining Particles 3 Months After Instillation
(Source: Reference 34)

In a study that will be quoted in later sections because of its carcinogenicity data, Heinrich et al[39] compared the chronic inhalation effects of coal oven flue gas mixed with pyrolysed pitch with those of diesel engine exhaust.

In this experiment, Wistar rats, NMRI mice and Syrian golden hamsters were exposed to diluted total exhaust, with particulate concentrations approaching 4 mg/m^3, for 19 hours/day, 5 days/week for a maximum of 2.5 years. Comparable groups of the same species of rodents were exposed to coal oven flue gas, which is rich in polycyclic aromatic hydrocarbons (PAH's). These emissions had 6 mg of particles/m^3 at least during the last three months of the 22-month exposure period. The schedule was averaged at 16 hours/day, 5 days/week.

The researchers wrote: *"The most remarkable difference between these two exposure groups was the particle load of the lungs. Only the diesel exhaust exposed lungs showed high deposits of soot particles combined with inflammatory changes, alveolar septal thickening and bronchiolar-alveolar hyperplasia. These effects correspond very well with the deterioration of the alveolar lung clearance mechanism, leading to a reduced particle elimination from the lungs."*

In comparison, practically negligible amounts of particle deposits were detectable after coal oven flue gas exposure and the lung clearance was not compromised. The heavy load of soot particles observed in diesel exhaust exposed lungs resulted in tissue reactions such as inflammation and fibrotic lesions. On the other hand, no obvious toxic effect could be ascertained in the lungs of rats inhaling the PAH-enriched coal oven flue

gas. The virtual absence of deposits after this latter exposure regimen was explained by the fact that the particulate matter consisted of condensed organic material which was easily dissolved, metabolized and eliminated from the lungs. However, the dose of chemical carcinogens appeared to be high enough to induce benign and malignant lung tumours.

Castranova et al[40] evaluated the response of rat alveolar macrophages to chronic inhalation of coal dust and/or diesel exhaust. Groups of male rats were exposed to: 1) 2 mg/m^3 respirable coal dust; 2) 2 mg/m^3 respirable diesel exhaust particulates; 3) 1 mg/m^3 each of respirable diesel exhaust particulates and coal dust; 4) filtered air (Control).

Coal dust was chosen because exposure to this material has been associated with the formation of lung lesions characteristic of coal miners pneumoconiosis. Bituminous coal was used and a four cylinder, four cycle Caterpillar diesel engine consuming No. 2 diesel fuel produced the diesel exhaust. The exposure atmospheres are described in Table 27. The animals were exposed 7 hours/day, 5 days/week, for 2 years.

Table 27

Atmospheric Concentrations of Major Chamber Components Over the 24-Month Exposure Duration

(Source: Reference 40)

Component	*Treatment*			
	FA	*CD*	*CD + DEP*	*DEP*
Respirable particles (mg/m^3)	NM*	2.1 ± 0.4**	2.0 ± 0.3	1.9 ± 0.3
CO_2 (%)	0.07 ± 0.02	0.08 ± 0.04	0.17 ± 0.06	0.16 ± 0.04
CO (ppm)	2.0 ± 0.9	2.1 ± 0.8	10.3 ± 2.0	10.5 ± 2.3
NO (ppm)	0.08 ± 0.13	0.08 ± 0.28	7.6 ± 2.8	7.8 ± 3.1
NO_2 (ppm)	0.06 ± 0.04	0.07 ± 0.05	1.5 ± 0.5	1.5 ± 0.5
SO_2 (ppm)	NM	0.003 ± 0.005	0.5 ± 0.3	0.6 ± 0.4
NH_3 (ppm)	0.5 ± 0.6	0.6 ± 0.7	0.4 ± 0.3	0.6 ± 0.8

Note: Airborne concentrations in the filtered air (FA) and coal dust chambers arose from the same ambient filtered/conditioned air supply and thus should be almost identical, except for the coal dust that was introduced. CD + DEP and DEP represent contributions from the same generation source with the baghouse filtration in the former instance the only variable. A continuous reading of the respirable particulate concentration was obtained within each exposure chamber by light scattering using a cyclone preseparator and a respirable aerosol monitor. Time-weighted averages of respirable dust concentrations (data shown: mean ± SD) were taken three times a day using a Mine Research Establishment gravimetric sampler (Casella London Limited, Model 113A) which measures respirable particulate concentration using a horizontal elutriator in series with a filter by criteria established by the British Medical Research Council. Readings from both methods were always in close agreement.

* NM, not routinely monitored. ** Mean ± SD

As can be seen in Table 28, the protein content, volume and yield of macrophages were monitored. The only statistically significant changes affected by the different exposure regimens are the following: cells from rats exposed to diesel exhaust were approximately 7% smaller than control and cell yield was increased by 184% after a two year exposure to coal dust. Protein content was not significantly affected by exposure.

Table 28

Properties[a] of Rat Alveolar Macrophages After Exposure for Two Years
(Source: Reference 40)

	Protein (mg/10^7 cells)	*Mean Cell Volume (μm^3)*	*Mean Cell Number (cells/rat)*
Control	2.7 ± 0.2	1798 ± 27	0.87 (± 0.08) × 10^7
Coal dust	2.8 ± 0.2	1727 ± 49	2.47 (± 0.73) × 10^7*
Diesel exhaust	3.6 ± 0.5	1664 ± 43*	1.06 (± 0.08) × 10^7
Diesel exhaust + coal dust	3.3 ± 0.2	1739 ± 36	0.99 (± 0.08) × 10^7

[a] Values are the means ± SEM of at least five experiments
* Significantly different from control ($p < 0.05$)

When lysosomal enzymatic activity was quantitated, β-N-Acetylglucosaminidase was the only enzyme of the three measured (the other two being acid phosphatase and β-galactosidase) that showed a significant increase in activity after exposure to diesel exhaust.

Macrophage activity, in the form of respiratory bursts, was measured by chemiluminescence. Exposure to coal dust resulted in a substantial increase in resting chemiluminescence indicting in a significant enhancement of macrophage activity. On the other hand, diesel exhaust depressed the phagocytic activity of these cells. Figures 24 and 25 illustrate these findings which are supported by similar results from Lewis et al(41).

From the data in reference 42, Vostal(6) estimated the approximate rate of particle accumulation per milligram·hour of exposure/m^3. It should be noted that, according to these data, the amount of particles in the lungs increased linearly with exposure. The calculations revealed that approximately 0.0008 mg of soot accumulated in the lungs per every mg·hr/m^3 of exposure when the inhaled concentrations were 3.5 mg/m^3 and higher.

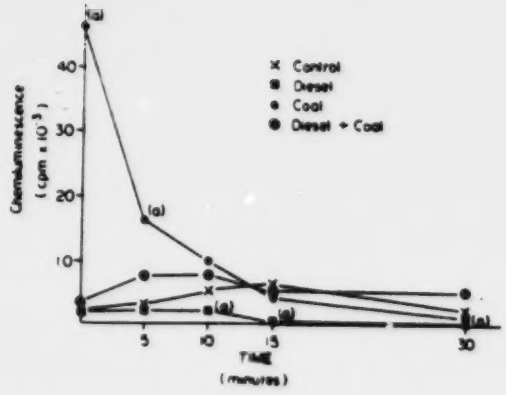


Figure 24

Resting Chemiluminescence by Alveolar Macrophages Obtained from Rats After Two Years of Inhalation Exposure to Filtered Air (control), Coal Dust (2 mg/m3), Diesel Exhaust (2 mg/m3), or Coal Dust (1 mg/m3) plus Diesel Exhaust (1 mg/m3).

Resting chemiluminescence was enhanced by the addition of 10-8 M luminol. Values are means of five separate experiments while (a) designates values designates values significantly different from control ($p<0.05$)

(Source: Reference 40)

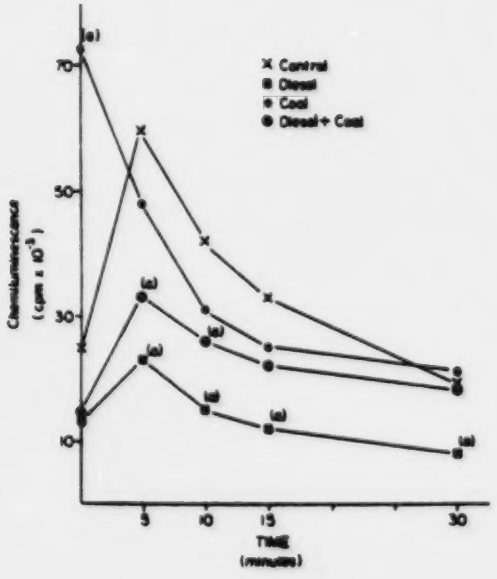


Figure 25

Chemiluminescence Generated by Alveolar Macrophages in Response to Zymosan (6 mg/mL).

Values are means of five separate experiments while (a) designates values significantly different from control ($p<0.05$).

(Source: Reference 40)

6. WHOLE ANIMAL, INHALATION STUDIES

After an examination of *in vitro* bioassay results and of general issues related to the clearance or accumulation of exhaust particles, the emphasis is now turned toward the tumourigenicity data obtained from animal studies. As will be discussed later, the value of data obtained from animals exposed through routes other than inhalation is severely limited. For this reason, the results from non-inhalation exposures were separated from those obtained from exposure by inhalation.

6.1 HEALTH EFFECTS OF SPECIFIC EXHAUST FRACTIONS

The different approaches taken by researchers allows the separation of experimental results so as to examine the health effects of each exhaust fraction separately. It should be noted that the classification of experimental results into the different categories was made quite arbitrarily. Focusing on the effects of each diesel exhaust component facilitated the task of determining exactly which of these was responsible for the observed toxicities.

In the following three sections, the health consequences of exposure to gases (versus total exhaust), "inert" particles, and PAH's will be examined.

6.1.1 Health Effects of Filtered and Unfiltered Emissions

Much information is available on the gaseous components of diesel engine emissions. In fact, their toxicity was abundantly documented even before that of diesel exhaust particulates or of the PAH's they contain. The 1984 edition of this report included relevant descriptions that are still accurate today(1).

Briefly, the health effects of the most important exhaust gases are the following:

Carbon dioxide (CO_2) represents no problem even at high concentrations if oxygen levels are maintained at 16 to 18%. Carbon monoxide (CO) and nitric oxide (NO) both reduce the oxygen carrying capacity of hemoglobin. However, mine ventilation, diesel exhaust control systems, and measured mine concentrations suggested that, except in rare instances, these gaseous pollutants are under control. On the other hand, nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) remain the gases of greatest concern from a health standpoint. They are both irritants that can cause various functional alterations in the respiratory system such as small airway hyperplasia and emphysematous-like responses.

The particulate matter emitted as a part of diesel exhaust has been implicated in comparatively more serious health effects. Indeed, as mentioned earlier and as Kawabata et al(34) wrote: "*The lung tumour formation due to the*

inhalation of diesel exhaust, consisting of both particles and gases, seems to be chiefly related to the particulate component."

By their design, the series of experiments summarized below have allowed to differentiate between the health effects of the gaseous and the particulate components of diesel engine exhaust: researchers have exposed some of their animals to total exhaust and some others to a filtered, particulate-free exhaust fractions to isolate the effects of gases from those of particulate. This approach allows the investigation of synergism or additivity of effects between the two exhaust fractions.

The Fraunhofer Institute[43,44] exposed three animal species to a single concentration of particulate-free and total exhaust. The study was conducted in three species of rodents, including male and female Syrian Golden hamsters, female NMRI mice and female SPF Wistar rats. The diesel exhaust source was a 40 kw 1.6 litre diesel engine operated in accordance with the US 72 test driving cycle using a fuel containing 0.36% sulphur. Three exposure groups were studied, including clean air (control), exhaust without particulate and total exhaust. The composition of the exhaust in the exposure chamber is shown in Table 29[44].

In addition to the basic study group, several subgroups which had been pretreated with benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DBahA), diethylnitrosamine (DEN), or dipentylnitrosamine (DPN) were included in the study in order to increase the baseline tumour incidence (Table 30).

Exposure was for 19 hours/day, five days/week throughout the lifetime of the animals. Particle-associated PAH's were measured on occasion giving values for airborne concentrations of 13 ng/m^3 benzo(a)pyrene, 21 mg/m^3 benzo(e)-pyrene, and 51 mg/m^3 benzofluoranthenes. Expressed as BaP content per particle mass the value was 3 µg/g which compares to 16 µg/g used in the EPA inhalation studies. The mass median aerodynamic diameter of the diesel particulate was 0.35 ± 0.10 µm.

Hamsters demonstrated no differences in body weight gain between treated and untreated groups. After 480 days of exposure, mice and rats receiving total diesel exhaust had significantly higher weight loss than the controls and the weight increase of exposed rats was retarded compared to controls. The median life span of the animals (the time period of exposure at which 50% of the animals were still alive) was not influenced by exposures in any of the three species. For hamsters the median lifetime was 75 to 80 and 80 to 90

weeks for female and male animals, respectively. Mice had median life spans of 85 to 95 weeks and rats 95 to 105 weeks.

After one year of exposure to unfiltered diesel exhaust the wet and dry lung weight of mice and rats increased (Figure 26). After two years of exposure the difference between initial and final lung weight amounted to a factor of 2 to 3. After 1 or 2 years of exposure hamsters demonstrated an increase of 50 and 70% respectively, in lung weights for animals exposed to total diesel exhaust.

Table 29

Mean ± SD Values for Exhaust Components Measured in the Exposure Chambers

(Source: Reference 44)

Component	*Control (clean air)*	*Exhaust Dilution (exhaust/exhaust + air) 1/17*	
		Exhaust Without Particles	*Total Exhaust*
CO (ppm)	0.16 ± 0.27	11.1 ± 1.92	12.5 ± 2.18
CO_2 (Vol. %)	0.10 ± 0.01	0.35 ± 0.05	0.38 ± 0.05
SO_2 (ppm)	---	1.02 ± 0.62	1.12 ± 0.89
NO_X (ppm)	---	9.9 ± 1.80	11.4 ± 2.09
NO (ppm)	---	8.7 ± 1.84	10.0 ± 2.09
NO_2 (ppm)	---	1.2 ± 0.26	1.5 ± 0.33
C_nH_m (ppm)	3.5 ± 0.29	5.2 ± 0.65	5.5 ± 0.69
CH_4 (ppm)	2.3 ± 0.17	2.4 ± 0.20	2.6 ± 0.19
C_nH_m without CH_4 (ppm)	1.3 ± 0.15	2.9 ± 0.50	3.1 ± 0.53
Particles (mg/m)	---	---	4.24 ± 1.42

Table 30

Experimental Plan and Number of Animals Used

(Source: Reference 44)

	Control (clean air)	*Exhaust Without Particles*	*Total Exhaust*
Hamsters (Female/Male)	96	96	96
Hamsters + DEN s.c. (4.5 mg/kg bw)	96	96	96
Hamsters + BaP i.tr. 20 × 0.25 mg	96	96	96
Hamsters (chronic toxicology)	96	96	96
Mice (Female)	96	96	96
Mice + BaP i.tr. 20 × 50 µg	64	64	64
Mice + BaP i.tr. 10 × 100 µg	64	64	64
Mice + DBahA i.tr. 10 × 50 µg	64	64	64
Mice (chronic toxicology)	96	96	96
Newborn mice (Female)	96	---	---
Newborn mice + DBahA 10 µg s.c.	96	96	96
Newborn mice + DBahA 5 µg s.c.	96	96	96
Rats (Female)	96	96	96
Rats + DPN s.c. 25 × 500 mg/kg bw	48	48	48
Rats + DPN s.c. 25 × 250 mg/kg bw	48	48	48
Rats (chronic toxicology)	96	96	96

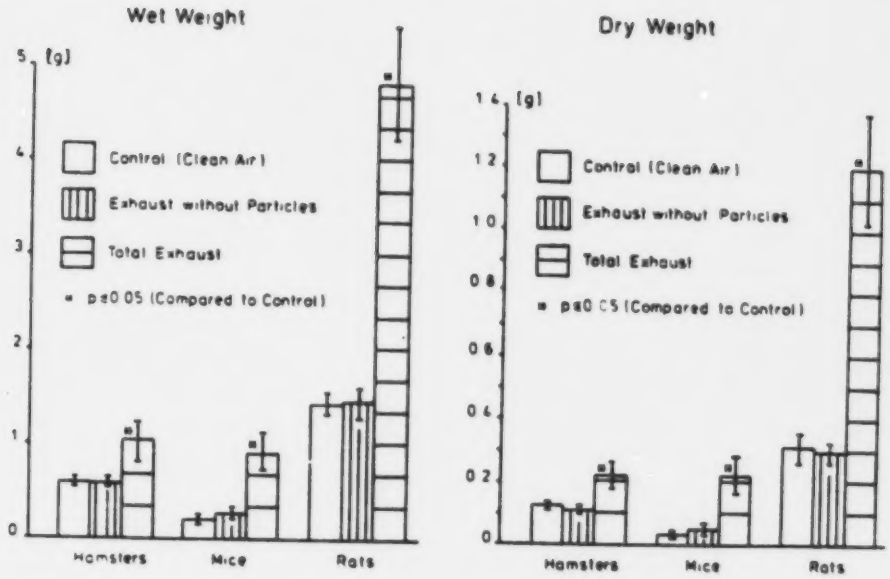


Figure 26

Wet and dry weight of lungs (n = 9) after 2 years of exposure
(Source: Reference 44)

Measurement of alveolar lung clearance was carried out in rats after 3, 8, 12 and 19 months of exposure and after 12 months in hamsters using a radioactive aerosol of $^{59}Fe_2O_3$. Diesel exhaust with particulate caused an insignificant decline in lung clearance after one year in hamsters. At that time the clearance half-life was 75 ± 40 days for exposed hamsters compared to 55 ± 17 days for the clean air controls.

The overall half clearance time of the control animals at all four measurement times was 49.5 ± 15 days. Rats exposed to diesel exhaust without particulate did not differ significantly in clearance rate to control animals. However, rats exposed to diesel exhaust containing particulate had significantly slower clearance times after only three months of exposure, with the clearance half-life increasing by approximately 2.5 times compared to controls. Interestingly, continued exposure did not further increase the clearance half-time

and, in fact, the values after 12 and 19 months were slightly lower than at 3 and 8 months Figure 27.

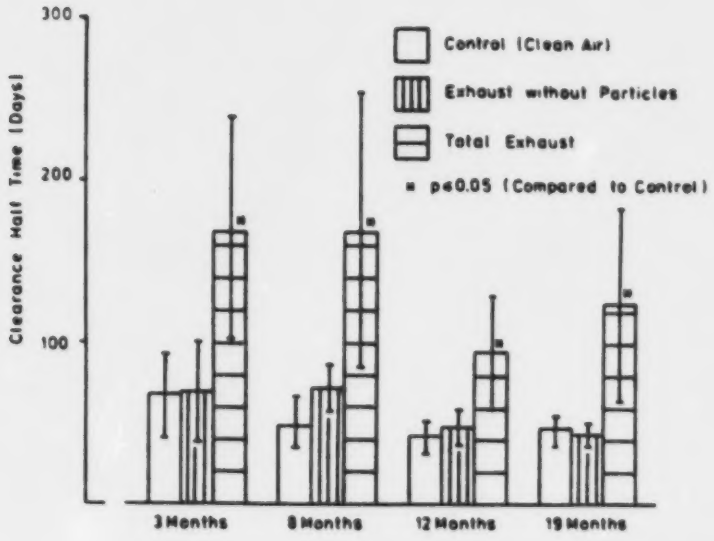


Figure 27

Clearance Half-time of a Test Aerosol ($^{59}Fe_2O_3$) in Rat Lungs After 3, 8, 12 and 19 Months of Exposure
(Source: Reference 44)

Physiological measurement of lung function in rats after two years of exposure provided the data shown in Table 31. Both compliance and resistance were significantly different in animals treated with total exhaust compared to control and animals receiving exhaust without particulate.

In hamsters, a significant increase in airway resistance and an insignificant change in lung compliance was measured after one year in animals receiving total exhaust. There was neither an indication of any increase or induction in lung tumours after a lifetime of exposure nor a significant increase in tumours of the upper respiratory tract. In animals receiving total diesel exhaust there was a qualitative increase in the incidence of animals with thickened septa, bronchiolo-alveolar hyperplasia and emphysematous lesions compared to controls. No differences were seen between control animals and those exposed to the filtered diesel exhaust.

Table 31

Lung Function Measurements in Rats (n = 14) After 2 Years of Exposure

(Source: Reference 44)

	Control (clean air)	*Exhaust Without Particles*	*Total Exhaust*
Tidal Volume (mL)	1.33 ± 0.28	1.51 ± 0.30	1.30 ± 0.15
Minute Volume (mL/min)	80.4 ± 13.54	90.7 ± 13.35	91.1 ± 12.83
Compliance (mL/cm H_2O)	0.281 ± 0.09	0.322 ± 0.07	0.190 ± 0.02[a]
Resistance (Res) (cm H_2O/mL/s)	0.544 ± 0.10	0.455 ± 0.08	0.628 ± 0.10[a]
Res.-Increase with ACH (cm H_2O/mL/s)	0.050 ± 0.049	0.050 ± 0.040	0.054 ± 0.059
Functional residual capacity (mL)	3.66 ± 0.64	3.58 ± 0.63	3.42 ± 0.58
Respiratory rate (breaths/min)		66 ± 8	

[a] $P \leq 0.05$ compared with control. ACH = Acetylcholine chloride

In mice, control animals showed a spontaneous tumour incidence of both benign and malignant types, of 13% by the end of the study. Lifetime exposure to diesel exhaust, with and without particulate, increased the tumour incidence to 32 and 31%, respectively (Table 32).

Table 32

Number and Percentage of Mice with Benign (adenomas) and Malignant (adenocarcinomas) Lung Tumours after a Maximum of 120 Weeks of Exposure

(Source: Reference 43)

Animals with:	*Control (n = 84)*	*Gas Phase (n = 93)*	*Total Exhaust (n = 76)*
Benign + Malignant Tumours	11≃13%	* 29≃31%	* 29≃31%
Benign Tumours Only	9≃11%	11≃12%	11≃15%
Malignant Tumours	2≃2.4%	* 18≃19%	* 13≃17%

The background incidence of malignant adenocarcinomas in control animals was 2.4% in mice, which increased to 19 and 17% in animals receiving exhaust without and with particulate, respectively. The incidence of multiple bronchiolo-alveolar hyperplasia was 64% in total diesel exhaust exposed animals, compared to 15% in particle-free exposed animals, and 5% in controls. Multifocal alveolar lipoproteinosis was noted in 71% of the mice exposed to total diesel exhaust, compared to 3 and 4% of the filtered diesel exhaust and control animals, respectively. Multifocal interstitial fibrosis occurred almost exclusively in mice exposed to total diesel exhaust.

In rats, no significant differences were noted in histological findings in the lungs of control and animals exposed to filtered diesel exhaust, although significant morphological changes occurred in animals exposed to a lifetime of total diesel exhaust as detailed in Table 33.

Table 33

Number of Histopathological Findings in the Rat Lung After a Maximum of 140 Weeks of Exposure

(Source: Reference 43)

Histological Findings	*Control (Clean Air)*	*Exhaust Without Particles*	*Total Exhaust*
Bronchiolo-Alveolar Hyperplasia	1	0	94
Metaplasia of the Bronchiolo-Alveolar Epithelium	0	0	62
Bronchiolo-Alveolar Adenoma	0	0	8
Squamous Cell Tumour	0	0	9
Number of Animals Exposed	96	92	95

In the group receiving total diesel exhaust, 15 out of 95 lungs exhibited a total of 17 tumours diagnosed as 8 bronchiolo-alveolar adenomas and 9 squamous cell tumours. Hyperplasia was seen in the lungs of 94 out of 95 rats, while metaplasia occurred in 62 animals. In 62 lungs the metaplastic lesions were diagnosed as nodular squamous cell metaplasias. Additional evidence of severe inflammatory changes included thickening septa, foci of macrophages and cholesterol crystals in the lungs of rats exposed to total diesel exhaust.

A classification scheme of squamous cell tumours is shown in Table 34.

Table 34

Classification Scheme of Squamous Cell Tumours that may Occur in Exposed Rat Lungs

(Source: Reference 44)

Classification		Behaviour	Cytology
1. Benign keratinizing cystic	*Cystic*	Slow peripherhal extension by air spaces	Regular, no atypia, mitoses absent or rare
2. Grade I carcinoma		Slight evidence of stromal invasion (peribronchial/ perviascular), possibly minimal scirrhous response	Borderline atypia occasional mitoses
3. Grade II carcinoma		Definite stromal invasion especially lymphatic or blood vascular, usually some scirrhous response	Mild atypia ± moderate mitotic index
4. Grade III carcinoma	*Malignant*	Invasion/destructive/scirrhous pleural/mediastinal spread	Moderate atypia + moderate-high mitotic index

No differences in the incidence of respiratory tract tumours were found in hamsters pre-treated with DEN and BaP, with or without exhaust particulate exposure. In mice, although increases in lung tumour rates were observed, no differences occurred as a result of exposure to total diesel exhaust. Subcutaneous DPN induced high lung tumour rates in control mice with most being malignant (Table 35).

At both DPN dosage levels exposure to total diesel exhaust resulted in a significant increase in the occurrence of squamous cell carcinomas, as well as a decrease in the incidence of papillomas in the upper respiratory tract, in rats exposed to diesel exhaust with and without particulate.

The data confirms the findings from other laboratories that the induction of respiratory tract tumours by long term diesel exhaust inhalation varies with the species. For example, virtually no effect of diesel exhaust and tumour incidence was found in Syrian Golden hamsters. In the case of female NMRI mice, because of a high spontaneous tumour rate, the evidence is weak that diesel exhaust induced a significant increase in tumour incidence. In female Wistar rats, in which there was almost no spontaneous tumours in untreated animals, unfiltered diesel exhaust resulted in the development of significant (16%) numbers of lung tumours which did not occur in animals exposed to filtered exhaust, thus

suggesting that diesel particulate was instrumental in the induction of rat lung tumours.

Table 35

Tumour Incidences in the Respiratory Tract of Rats Treated with Two Different Doses of Diphenylnitrosamine (DPN)

(Source: Reference 43)

		+ 6.25 g DPN/kg bw					
		Control (Clean Air)	n	Exh. Without Particles	n	Total Exhaust	n
Lung	All Tumours	84.8%	46	67.4%	46	83%	47
	Squamous Cell Ca	4.4%		4.4%		*46.8%	
Upper Respiratory Tract	Benign Tumours Only	26.1%	46	*4.4%	45	*8.7%	46
	Malignant Tumours	2.2%		---		---	
		+ 12.5 g DPN/kg bw					
		Control (Clean Air)	n	Exh. Without Particles	n	Total Exhaust	n
Lung	All Tumours	93.8%	48	89.6%	48	89.6%	48
	Squamous Cell Ca	16.7%		14.6%		*31.3%	
Upper Respiratory Tract	Benign Tumours Only	47.9%	48	*22.9%	48	*14.6	48
	Malignant Tumours	8.3%		12.5%		16.7%	

* $p \leq 0.05$ (compared to control)

Overall, the data indicate that the rat lung is especially sensitive to heavy deposits of particulate matter as demonstrated by a reduction in pulmonary clearance and the induction of tumours as compared to other species. Given that particulate exposure in these studies were high (4 mg/m^3), and knowing that lung clearance mechanisms are compromised much more markedly at high than at low soot concentrations, it is difficult to extrapolate the data to environmental levels of diesel particulate.

A related study came from Batelle in Geneva. Brightwell et al[45] recently published the results of a chronic inhalation study in rats and hamsters exposed to exhaust from a 1.5 litre Volkswagon engine run on the US-72 Federal Test Procedure driving cycle. A summary of the exposure conditions is given in Table 36.

Table 36

Exposure Conditions
(Source: Reference 45)

	CO (ppm)			NO_X (ppm)			Part. (mg/m^3)		
	L	M	H	L	M	H	L	M	H
Controls	--	--	1	--	--	0.1	--	--	0.2
Diesel	3.6	10.7	32	0.9	2.7	8	0.7	2.2	6.6
Filtered Diesel	3.6	10.7	32	0.9	2.7	8	0.2	0.2	0.2

Animals used in the study included Fisher 344 rats and Charles River Syrian hamsters, which were exposed for 5 - 16 hour periods per week for two years. Table 37 provides a breakdown of the number of animals in each experimental group. The diethylnitrosamine (DEN) treated hamsters received a 4.5 mg/kg subcutaneous injection of DEN three days before beginning exposure. Surviving hamsters were sacrificed at the end of two years, but rats surviving to the end of that exposure period continued a six month recovery period without exposure to exhaust emissions.

Table 37

Number of Animals in Each Experimental Group and Scheduled Sacrifice Times
(Source: Reference 45)

	Number of Animals*		
	Rats	Hamsters	DEN-Hamsters
At start of exposure	144	208	104
Interim sacrifices:			
6 months	16	16	--
12 months	16	--	--
16 months	--	16	--
18 months	16	--	--
24 months	16	--	--
Final sacrifice**	80	176	104

* In each group or sub-group the animals were evenly divided between males and females

** The numbers in this row include both those animals surviving to final sacrifice and those dying, or sacrificed when moribund, during the experiment

All animals that died during, or were sacrificed at completion of, the study were subjected to necropsy and histopathological examination of major organs and respiratory tract tissues, including nasal passages, larynx, trachea and lungs for all high dose and control animals. As well, histological examination was completed on the respiratory tract of all DEN-treated hamsters as well as medium and low dose diesel and particulate-free diesel exposed animals. All suspected tumours were subjected to histopathological examination. Blood chemistry and hematology, as well as cardiovascular function and respiratory measurements were carried out on various subgroups of animals, at various times during the study.

Body weights of rats exposed to the medium and high doses of diesel exhaust had significantly reduced body weights at 24 months, with the greatest decrease observed in the high dose diesel exhaust group, in which the body weights were reduced by 23% for males and 31% for females, as compared to control animals. Chemistry measurements indicated that some animals had liver damage. The relative significance of changes compared to control groups is given in Tables 38 and 39. Absolute values were not published.

The major changes in rats included a decrease in glucose, cholesterol and triglycerides and an increase in BUN. In hamsters a decrease of potassium, LDH, HBDH and aspartate aminotransferase and an increase in albumin, GTP were measured for diesel exhaust exposed animals. Although, not clearly presented, it is assumed that these data reflect the changes in the animals receiving unfiltered diesel exhaust. The major hematological changes seen in diesel exposed rats included increased RBC, hemoglobin and hematocrit as well as an increase in WBC due to segmented neutrophils (Table 40). Relatively few hematological changes were noted in hamsters (Table 41).

Although details were not presented, it was indicated that the respiratory physiology measurements showed no treatment-related changes in the high dose level hamsters, whereas in rats a large number of pulmonary parameters showed statistically significant differences as a result of exposure to diesel exhaust. The changes in rats were consistent with the presence of dose-related obstructive and restrictive airways disease in animals exposed to high concentrations of diesel exhaust.

Table 38

Significant Changes in the Blood Chemistry of Rats Exposed to Diesel Engine Exhaust Emissions[1]

(Source: Reference 45)

Parameter	Duration of Exposure (months)	Diesel	
		Male	Female
Glucose	18	↓	--
	24	↓	↓
Blood urea nitrogen	18	--	--
	24	↑↑	↑↑↑
Total proteins	18	--	--
	24	--	↓
Triglycerides	18	--	--
	24	--	↓↓
Cholesterol	18	↓↓↓	
	24	↓↓	
Alkaline Phosphatase	18	--	--
	24	↑	↓↓
Gamma-glutamyl transpeptidase	18	--	--
	24	--	↑↑↑
Creatinine phosphokinase	18	--	--
	24	--	--
Lactate dehydrogenase	18	--	--
	24	--	--
α-hydroxybutyric	18	--	--
	24	--	--
Cholinesterase	18	--	--
	24	↓↓	--
Alanine aminotransferase	18	--	↑↑
	24	--	--
Aspartate aminotransferase	18	--	↑↑
	24	--	↑

[1] The data given are for the 18 and 24 month exposure periods only. In each case the data have been compared with the relevant control data (t-test) and a statistically significant difference is shown as an increase (↑) or decrease (↓) compared to controls. The number of arrows indicates the degree of significance: ↑ = $p<0.05$; ↑↑ = $p<0.01$; ↑↑↑ = $p<0.001$.

Table 39

Significant Changes in the Blood Chemistry of Hamsters Exposed for Sixteen Months to High Concentrations of Diesel Engine Exhaust Emissions[1]

(Source: Reference 45)

Parameter	Diesel	
	Male	Female
Glucose	--	--
Albumin	--	↑
Cholesterol	--	--
Sodium	--	--
Potassium	--	↓
Gamma-glutamyl transpeptidase	↑↑↑	↑↑
Lactate dehydrogenase	--	↓
α-hydroxybutyric	--	↓
Cholinesterase	--	--
Aspartate aminotransferase	--	↓

[1] The data given are for the 16 month exposure periods only. In each case the data have been compared with the relevant control data (t-test) and a statistically significant difference is shown as an increase (↑) or decrease (↓) compared to controls. The number of arrows indicates the degree of significance: ↑ = $p<0.05$; ↑↑ = $p<0.01$; ↑↑↑ = $p<0.001$.

Table 40

Significant Changes in the Hematology of Rats Exposed to High Concentrations of Diesel Engine Exhaust Emissions[1]

(Source: Reference 45)

Parameter	Duration of Exposure (months)	Diesel	
		Male	Female
Red blood cell count	18	↑	↑↑
	24	--	↑↑↑
Haemoglobin	18	↑↑↑	↑↑↑
	24	--	↑↑↑
Haematocrit	18	↑↑	↑↑↑
	24	--	↑↑↑
Mean corpuscular volume	18	--	--
	24	--	--
Prothrombin time	18	↑↑	--
	24	--	↑
White blood cell count	18	--	↑
	24	↑	↑↑↑
Segmented neutrophils	18	↑	↑↑
	24	--	↑↑
Lymphocytes	18	↓	↓
	24	--	↓

[1] The data given are for the 18 and 24 month exposure periods only. In each case the data have been compared with the relevant control data (t-test) and a statistically significant difference is shown as an increase (↑) or decrease (↓) compared to controls. The number of arrows indicates the degree of significance: ↑ = $p<0.05$; ↑↑ = $p<0.01$; ↑↑↑ = $p<0.001$.

Table 41

Significant Changes in the Hematology of Hamsters Exposed for Sixteen Months to High Concentrations of Diesel Engine Exhaust Emissions[1]

(Source: Reference 45)

Parameter	Diesel	
	Male	Female
Red blood cell count	--	--
Haemoglobin	--	↑
Haematocrit	--	↑↑
White blood cell count	--	--

[1] The data given are for the 16 month exposure periods only. In each case the data have been compared with the relevant control data (t-test) and a statistically significant difference is shown as an increase (↑) or decrease (↓) compared to controls. The number of arrows indicates the degree of significance: ↑ = $p<0.05$; ↑↑ = $p<0.01$.

In diesel exhaust exposed rats the left ventricular dP/dt maximum values were significantly lower than in controls, being 2340 ± 595 and 3040 ± 388 mm Hg/sec in diesel exposed and control animals, respectively. Diesel exposed rats also had significantly greater right ventricular/heart weight ratios than controls,as well as significantly greater right ventricular weight/body weight and total heart weight/body weight ratios. A statistically significant increase in lung weight was measured in both rats and hamsters exposed to diesel exhaust, compared to control animals.

The incidence of tumour-bearing rats in diesel exhaust exposed animals was 14/144 (10%) and 55/143 (138%) for the medium and high dose levels, respectively. The incidence was dose-related and was higher in females (11/72 [15%] and 39/72 [54%] for medium and high exposure levels) than in males (3/72 [4%] and 16/71 [23%] for medium and high doses). No increase in the incidence of primary lung tumours was seen in the control or low dose group.

In the non-DEN treated hamsters respiratory tumours were rare and not related to treatment. In the DEN pretreated hamsters there was an increase in tracheal papillomas in both the treatment and control groups, compared with the incidence in non-DEN treated animals. However, there was no significant effect related to diesel exhaust exposure. The hematological changes seen in rats exposed to diesel exhaust was probably a result of hypoxia. These animals also showed marked restrictive and obstructive airways disease, which could also be due to hypoxia. Cardiovascular findings in exhaust exposed rats were consistent with the presence of obstructive lung disease.

The incidence of lung tumours in diesel exhaust exposed rats was dose related to high levels of unfiltered diesel exhaust exposure and was considered to be the result of pulmonary deposition and retention of diesel exhaust particulate, since no increase in tumour incidence was seen in rats exposed to filtered diesel exhaust.

Iwai et al[46] also carried out a long-term inhalation experiment to try to prove that diesel exhaust is responsible for tumour induction in rats. A 2.4 L (small) truck diesel engine run at 80% engine load and 1000 rpm was used as a source of diesel exhaust, with 10 fold dilution. The rationale for this condition was that it gave the highest concentration of particulate with low NO_2 and SO_2. A number of the exposure chambers were equipped with HEPA filters in order to provide particulate-free diesel exhaust. Fischer 344 female rats were divided into three groups of 24 rats each, one of which received diluted whole exhaust, another diluted, filtered exhaust and the third was a control group which

received clean air. Exposure was carried out for 8 hours per day, seven days a week for 24 months. The dilution tunnels and chamber had pollutant concentrations as follows:

NO_X	30.9 ± 10.9 ppm
NO_2	1.8 ± 1.8 ppm
SO_2	13.1 ± 3.6 ppm
CO	7.0 ± 1.4 ppm
Particulate	4.9 ± 1.6 mg/m^3

After about six months of exposure the body weight gains of animals receiving particulate exhaust were significantly less than those of the control or filtered exhaust animals. Compared to control animals, the lung to body weight ratio increased more than 2-fold after 12 months exposure to whole exhaust, and roughly 3 times after 24 months. By 24 months animals receiving filtered exhaust had a significantly greater lung-to-body weight ratio than controls, but the differences were much smaller than for those receiving whole exhaust. For example, the ratio for control animals was 3.1 ± 0.4 compared to 4.6 ± 0.5 and 9.2 ± 1.9 for animals receiving filtered exhaust and whole exhaust, respectively. The particulate content of the lungs increased over the two years at each observation point, with the numbers of particles engulfed in macrophages increasing markedly. Fibrous thickening of the alveolar walls and mass cell infiltration occurred with epithelial hyperplasia and pigment-laden macrophages being observed in animals exposed to diesel particulate. Adenocarcinoma and squamous or adenosquamous carcinomas were observed in animals treated with whole exhaust Table 42[46].

Among the 19 rats exposed to whole exhaust for 2 years 8, or 42.1%, had lung tumours of which 5 (26.3%) were malignant. Malignant lymphomas of the spleen occurred in significantly more of the animals on whole or filtered exhaust than for controls (Table 43). Malignant lymphomas were found at 30 months in control animals whereas they appeared after 15 months of exposure in the experimental groups.

After two years of exposure, 8.2% of control rats were found to have tumours of other organs, whereas 25% and 29.1% of the animals in the filtered exhaust and whole exhaust groups, respectively, were found to have some kind of a tumour, other than in the lung or spleen (Table 44).

Table 42

Lung Tumour in Two-Year Exposed Rats

(Source: Reference 46)

	Incidence of Lung Tumours		
	Died within 2 years	2-year-exposed	2-year-exposed + 6 months' observation
Control n = 24	0/2	0/15 1/22 (0/22	1(0)/7 4.5% 0%)
Filtered Exhaust n = 24	0/8	0/13 0/16	0/7 0%
Whole Exhaust	0/5	4(2)/14 8/19 (5/19	4(3)/5 42.1%** 26.3%)*

** Significant in $p<0.01$ (with Yates' correction), compared to control

* Significant in $p<0.05$ (with Yates' correction), compared to control

No. in parenthesis: incidence of malignant tumours

Table 43

Malignant Lymphoma of Spleen With or Without Leukemia in Two-Year-Exposed Experiment

(Source: Reference 46)

	Incidence of Malignant Lymphomas			
	Total	*Died within 2 years*	*2-year-exposed*	*2-year-exposed + 6 months observation*
Control	2/24 8.2%	0/2	0/15	2/7
Filtered Exhaust	9/24 37.3%	5/8	3/13	1/3
Whole Exhaust	6/24 25.0%	2/5	1/14	3/5

Control: filtered exhaust $p<0.05$ (with Yates' correction)

Control: filtered + whole exhaust $p<0.05$

Table 44

Tumours in Other Organs in Two-Year-Exposed Experiment

(Source: Reference 46)

	Incidence of Tumours			
	Total	*Died within 2 years*	*2-year-exposed*	*2-year-exposed + 6 months observation*
Control	2(0)/24 8.2%	0/2	0/15	2(0)/7
Filtered Exhaust	6(0)/24 25.0%	2(0)/8	4(0)/13	0/3
Whole Exhaust	7(2)/24 29.1%	1(1)/5	3(0)/14	3(1)/5

Control: filtered exhaust $p<0.05$ (with Yates' correction)
Control: filtered + whole exhaust $p<0.05$
Numbers in parentheses: malignant tumours

Type II hyperplasia of the alveolar epithelium and respiratory bronchiolar epithelium were prominent reactions in diesel particulate exposed animals as a result of particulate deposition. These changes did not occur in the filtered exhaust group, which indicates that NO_2 and SO_2 do not produce obvious hyperplasia of the airway epithelium. The occurrence of lymphomas and leukemia in Fischer 344 control rats has been reported as ranging from 8.3 - 18.8%, which is in keeping with control values in the present study of 8.2%. Therefore, the 25 and 29% tumour incidence in the two experimental groups appears to be statistically significant.

6.1.2 Health Effects of Inert Particles

The exposure regimen of the studies presented above allowed the identification of the toxicity of the gaseous components of diesel exhaust and to separate them from that of the particulate fraction. However, the results from whole exhaust do not allow the differentiation between the health effects of the carbonaceous components of soot and the hundreds of compounds it contains, some of which are known mutagens and carcinogens.

Diesel exhaust particulate (DEP) is composed primarily of "soot" or carbon particles. Physically, they are aggregates of small spherical sub-particles. The individual primary particles have a diameter of 10 to 80nm and the agglomerates range 5 to 10 μ in size. Approximately 95% of the aggregate-particles are less than 1 μm in diameter and, because those below 0.5 μm are essentially 100% respirable, DEP's are largely respirable(1).

Researchers theorized that DEP's may contribute to the health consequences of whole exhaust by irritating respiratory tract epithelium. The results of the experiments that follow will attempt to determine the extent to which the carbonaceous and supposedly inert components of emitted particles are responsible for observed toxicities.

Vostal(6) has summarized the results of several experiments. He reported that tumours were produced in 11% of animals exposed to inhalable coal dust(47); that 4 to 25% malignant lung tumours were observed after inhalation of titanium dioxide and titanium tetrachloride hydrolysis products(48,49); that up to 25% tumours were found in animals exposed to dust from shale; and, finally, that solvent-refined coal solids, when inhaled, produced respiratory cancer in more than 55% of exposed animals. The experimental conditions of these studies were considered comparable to regular diesel exhaust inhalation investigations except for the use of higher exposure concentrations and of different particle types.

Wolff et al(4) investigated the extent to which the inhalation of diesel engine exhaust or carbon black lead to pulmonary inflammation and genotoxicity. Male F344/N rats were exposed to diluted diesel engine emissions or to carbon black 7 hours/day, 5 days/week for 12 weeks. The exposure concentrations were close to the target values of 3.5 and 10.0 mg/m^3. The particulate phases of the two exposures were similar in particle size distributions and, consequently the resultant lung burdens were comparable. As can be seen in Figure 27, lung burdens and clearance of carbonaceous particles were similar at each exposure concentration.

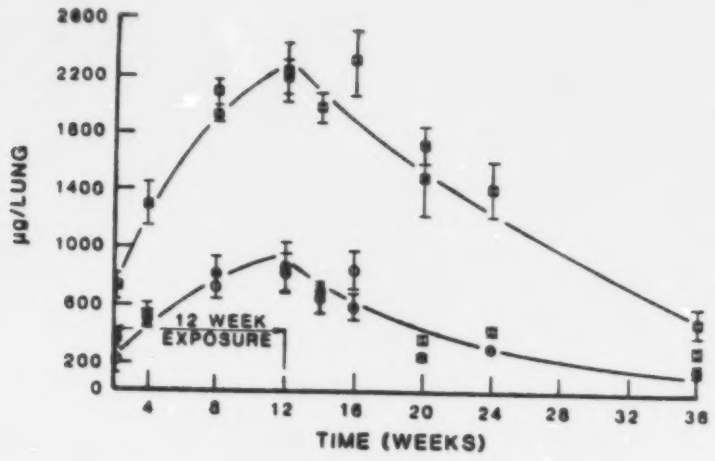


Figure 27

Lung Burdens of Particulates During and After the 12-Week Exposure to 3.5 mg/m³ (0) and 10 mg/m³ (□) diesel soot, and 3.5 mg/m³ (●) and 10 mg/m³ (■) carbon black. First-order clearance half-times following the end of exposure were 90 ± 31 days and 103 ± 25 days for the 3.5 mg/m³ and 10 mg/m³ concentrations of diesel soot, and 51 ± 9 days and 95 ± 22 days for the 3.5 mg/m³ and 10 mg/m³ concentrations of carbon black, respectively

(Source: Reference 4)

After 12 weeks of exposure to 10 mg/m³ of each emission, a mild inflammatory response, and significant increases in polymorphonuclear neutrophils and acid proteinase were observed. Because responses were comparable after exposure to diesel engine exhaust and to carbon black it was concluded that gases and vapours in the exhaust atmosphere, as well as the organic compounds associated with the particles, had a minor influence on lung inflammation. No significant inflammatory changes were associated with 12 week exposure to 3.5 mg/m³ of exhaust emissions or carbon black.

DNA adducts, which were monitored as a measure of genotoxicity, were found in all respiratory tract tissues for all exposure modes. The higher number of adducts present in parenchymal tissue, an area where tumours were observed previously in rats exposed to diesel exhaust, suggested a possible relationship between adducts and tumour formation. The levels of adducts were higher in rats exposed to diesel exhaust than to carbon black.

This last observation could be cited as evidence for the possibility that "non-carcinogenic" particles may exert a tumourigenic effect through the genetic mechanism, even though response was higher in diesel particulate exposed animals.

A study conducted by Lewis et al[41] used exhaust that was water scrubbed and, consequently, cannot be directly compared with results from untreated exhaust exposure, since water quenching would prevent the condensation of many high boiling exhaust constituents on soot nuclei (Paul Mogan, personal communication)[22]. Nevertheless, the results are included for comparison. Fischer 344 rats and Cynomolgus monkeys were exposed to various conditions of diesel exhaust, with or without added coal dust, for 7 hours a day, five days a week for 24 months[41,50]. A summary of the groups included in the study is shown in Table 45[41].

Table 45

Experimental Design Denoting the Treatment Groups, Particulate Exposures, Animal Allocations and Exposure Regimen[a]
(Source: Reference 41)

Treatment[b] Group	Particulate Concentration	Rats		Male Monkeys
		Male	Female	
FA[c]	0	72 144	72 0	15
CD	2 mg/m^3	72 144	72 0	15
DE	2 mg/m^3	72 144	72 0	15
DECD	1 mg/m^3 + 1 mg/m^3	72 144	72 0	15

a 7 hours/day, 5 days/week for 24 months
b FA-clean air, control; CD-coal dust; DE-diesel exhaust; DECD-diesel exhaust plus coal dust
c Three chambers per treatment; one housed male rats; the second male and female rats; and the third, monkeys

Diesel exhaust was generated by a Caterpillar 7 litre, naturally aspirated engine operated through a simulated 8 mode load/haul/dump cycle of which the idling mode constituted about 60% of the total cycle time of 10 minutes. The exhaust was diluted 27:1 and passed through a Wagner water scrubber prior to entering the exposure chambers.

Table 46[41] gives a summary of the pollutant concentration in the various experimental exposure chambers. Seven PAH's were quantitated in the respirable fraction from each dust chamber (Table 47).

Table 46
24-month Mean Concentrations of Airborne Components (Three Chambers per exposure atmosphere)
(Source: Reference 41)

Component	Unit	FA	CD	DE	DECD
Total Particles	mg/m³	--[a]	4.98 ± 0.82[b]	--	3.23 ± 0.60
Resp. Particles	mg/m³	--	2.00 ± 0.41	1.95 ± 0.25	2.01 ± 0.30
CO_2	%	0.08 ± 0.02	0.09 ± 0.05	0.20 ± 0.06	0.20 ± 0.07
CO	ppm	2.2 ± 0.9	2.2 ± 0.9	11.5 ± 3.1	10.9 ± 2.8
NO	ppm	0.08 ± 0.14	0.08 ± 0.29	8.7 ± 3.6	8.3 ± 3.2
NO_2	ppm	0.06 ± 0.04	0.06 ± 0.05	1.5 ± 0.5	1.6 ± 0.5
THC (cold)[c]	ppm C	4.1 ± 1.9	--	7.5 ± 2.2	7. 4 ± 2.0
THC (hot)	ppm C	--	--	--	8.3 ± 1.9
SO_2	ppm	--	0.01 ± 0.07	0.81 ± 0.38	0.61 ± 0.29
NH_3	ppm	0.52 ± 0.28	0.57 ± 0.52	0.64 ± 0.71	0.48 ± 0.55
Tot. Al. Aldyh[d]	ppm	0.02 ± 0.01	0.02 ± 0.01	0.12 ± 0.06	0.12 ± 0.05
SO_4^{2-}	µg/m³	--	16.8 ± 17.9	29.0 ± 24.9	42.3 ± 33.8
NH_4^+	ppb	--	6.5 ± 14.3	27.0 ± 30.7	16.5 ± 23.3
Acrolein	ppb	3.0 ± 3.3	6.2 ± 4.7	60.2 ± 24.5	57.8 ± 20.5
Acetaldehyde	ppb	1.5 ± 3.5	0.9 ± 2.5	38.7 ± 15.3	37.7 ± 14.0
Formaldehyde	ppb	7.6 ± 3.5	7.4 ± 4.1	38.3 ± 23.0	37.4 ± 26.6
Silica	µg/m³	ND[e]	21.9 ± 10.4	ND	ND
Silicon	µg/m³	NE	22.1 ± 10.3	ND	ND

[a] Not measured; [b] Mean ± SD; [c] Total Hydrocarbons; [d] Total aliphatic aldehydes;
[e] ND = not detected; limits of detection for silica and silicon were 12 µg/m3 and 11 µg/m3, respectively

Table 47
Concentrations of Polycyclic Aromatic Hydrocarbons in Diesel and/or Coal Respirable Particles (ng/m³)
(Source: Reference 41)

Component	FA	CD	DE	DECD
Benzo(a)anthracene	---[a]	3.2 ± 2.2[b]	19.6 ± 9.9	11.2 ± 5.2
Benzo(k)fluoranthene	---	---	5.6 ± 2.3	3.6 ± 2.4
Benzo(a)pyrene	---	---	13.5 ± 6.8	10.2 ± 6.5
Fluoranthene	---	26.5 ± 11.5	139.3 ± 98.1	67.5 ± 52.4
Pyrene	---	32.3 ± 15.1	123.4 ± 72.2	60.0 ± 36.6

[a] See text; [b] Mean ± SD

In rats, no significant differences in functional residual pulmonary capacity, airway resistance and alveolar morphometry were observed following one or two years of exposure.

Monkeys were evaluated every six months throughout the two year exposure period for pulmonary compliance, resistance, static and dynamic volumes, diffusing capacity, distribution of ventilation and maximal ventilatory performance. No responses were found that were consistent with restrictive lung disease. Obstructive responses, as measured by reduced flow were found in the diesel exposed monkeys (Table 48).

Table 48

Statistically Significant Pulmonary Function Test Results in Monkeys Exposed for 24 Months to Either Coal Dust, Diesel Exhaust, or a Combination of Both
(Source: Reference 41)

Test	Time				P-Value[a]
	6 Months	12 Months	18 Months	24 Months	
FEV_1[b]				DECD>CD	P = 0.009
PEF		FA>CD			P = 0.04
FEF-25			FA>DE	DECD>CD	P = 0.09
FEF-50		FA>CD			P = 0.03
FEF-50/FVC			FA>DE		P = 0.02
FEF-40/TLC		FA>DE	FA>DE	FA>DE	P = 0.05

a Group Comparisons (ratio to baseline x 100) were calculated using a Kruskall-Wallis test followed by pairwise comparisons. N = 15 for all groups except CD where N = 14.

b FEV1 = forced expiratory volume at 1 second
PEF = peak expiratory flow
FEF-25 = forced expiratory flow at 25% of vital capacity
FEF-50 = forced expiratory flow at 50% of vital capacity
FEF-50/FVC = forced expiratory flow at 50% of vital capacity/forced vital capacity
FEF-40/TLC = forced expiratory flow at 40% of total lung capacity/total lung capacity

Dust exposed monkeys had statistically obstructive lower expiratory flow rates after one year of exposure, which was not statistically significant at two years. Interestingly, in examining the particle content of lungs from exposed rats it was found that in animals exposed to diesel exhaust the quantity of diesel particulate increased with time, whereas those exposed to coal dust increased little over the last 12 months of the exposure period.

After 24 months of exposure alveolar macrophages from exposed rats were assayed for cell numbers, cell volume, cell morphology and biochemical indices, or treated for measurement of oxygen consumption and chemiluminescense. Exposure to coal dust and/or diesel exhaust had little effect on oxygen consumption, membrane integrity, lysosomal enzyme activity or protein content of alveolar macrophages. However, exposure to coal dust resulted in increased macrophage yield and enhanced chemiluminescense. As well, the cell membrane had increase cellular spreading and surface ruffling. The alveolar macrophages from diesel exhaust exposed animals demonstrated depressed chemiluminescense and decreased ruffling of the cell membrane.

At various times during the exposure schedule rat lungs were examined histopathologically. In rats exposed to diesel exhaust and/or coal dust, the pleural surfaces of the lungs showed black deposits of pigment which were concentrated in the lymphatics located at the junction of the lobular septa and pleura. No evidence of focal emphysema was seen in any of the exposure groups. At 24 months large numbers of particles within phagolysosomes of alveolar macrophages were observed using electron microscopy. Macrophages demonstrated degenerative changes which were notable in lungs from diesel exposed rats. Type II cell hyperplasia was observed in the alveolar epithelial lining. In monkey lungs no relationship between particulate exposure and fibrosis, focal emphysema or inflammation were observed.

Gross and histopathologic examination was performed on a large number of rats who survived to the termination of the study or who died throughout the exposure period. There were no differences in survival rates between treatments, chambers or sexes. For 50 tissues examined no differences in the incidence of neoplasia was observed and the values were similar to those reported for control groups in other studies. The authors concluded that the study provided evidence that diesel exhaust at respirable airborne concentrations of 2 mg/m^3 is more hazardous to health than its mining counterpart of 2 mg/m^3 respirable coal dust.

Mauderly et al[51] studied the health effects of unretorted (raw) or retorted (spent) oil shale dusts, and that of diesel exhaust. Each material was either used alone or in diesel- shale combinations. For the experiment, male and female F344/Crl rats were exposed to the conditions detailed in Table 49 for 7 hours/day, 5 days/week for up to 30 months. The experimental conditions did not significantly affect the animal's body weight and survival (Table 50).

Table 49

Exposure Atmospheres During 30 Months of Exposure[a]

(Source: Reference 51)

Constituent (units)	Control		Diesel		Raw Shale		Raw Shale and Diesel		Spent Shale		Spent Shale and Diesel	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Particles (mg/m^3)	0.05	0.002	3.5	0.02	5.1	0.06	8.5	0.04	5.0	0.04	8.6	0.05
Carbon monoxide (ppm)	1.1	0.07	12.9	0.5			13.2	0.5			12.7	0.5
Carbon dioxide (ppm)	1820	40	3080	50			3060	80			3010	60
Hydrocarbon vapors (ppm)	1.1	0.1	3.1	0.2			2.8	0.2			2.7	0.1
Nitrogen oxides (ppm)	0.1	0.02	4.6	0.2			4.9	0.2			4.5	0.2
Ammonia (ppm)	0.5	0.06	0.4	0.06			0.4	0.06			0.4	0.05

[a] Data are means and standard errors of 127 weekly values.

Disturbances in the concentrations of airway fluid constituents indicated chronic inflammation, cell damage and restricting of lung tissue. Inflammatory response was demonstrated by increased leukocyte counts. These parameters were affected to a decreasing degree by the combined exposures, diesel exhaust, and shale-only groups, respectively. The mass and density of excised lungs were increased in the combined exposure and diesel only groups. The only significant change was in the RS-D group, although non-significant changes in descending severity were seen in SS-D, D and RS and SS groups.

It was concluded that the health effects resulting from exposure to both diesel exhaust and oil shale dust were more than additive.

Table 50

Health Effects in Rats Exposed Chronically for Up to 30 Months to Oil Shale Dusts and Diesel Exhaust, Alone and in Combination

(Source: Reference 51)

Parameter (units)	n per group	Control		Diesel		Raw Shale		Raw Shale and Diesel		Spent Shale		Spent Shale and Diesel	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body Weight (BW) at 24 Months													
Male (g)	29-36	370	7	373	7	383	7	380	7	387	6	375	7
Females (g)	34-40	265	5	262	5	270	6	256	4	271	5	259	5
Median (50%) Survival (Life Table Analysis)													
Males (days of age)	45-50	939	16	937	6	914	13	915	13	936	8	935	24
Females (days of age)	41-46	961	16	996	16	917	13	950	24	940	24	999	14
Airway Fluid Constituents at 30 Months													
Total leukocytes (10^6/mL)	6-8	1.5	0.4	2.7	0.4	1.6	0.1	5.5	1.6	1.9	0.2	9.5[a]	1.1
Lactate dehydrogenase (mIU/mL)	6-8	81	14	255	36	65	9	456	79	99	14	446	51
Glutathione reductase (mIU/mL)	6-8	6.0	1.8	10.3	1.9	4.2	0.8	21.3[a]	2.9	5.1	1.0	17.5[a]	2.2
Beta glucuronidase (mIU/mL)	6-8	0.2	0.03	2.3	0.7	0.3	0.1	5.8[a]	1.6	0.3	0.1	6.1[a]	0.8
Acid proteinase (Units/mL)	6-8	32	2	88[a]	5	49[a]	3	105[a]	3	55[a]	3	107[a]	3
Total Protein (mg/mL)	6-8	0.19	0.04	0.36[a]	0.03	0.22	0.02	0.60[a]	0.10	0.25	0.03	0.75[a]	0.08
Lung Weight and Density at 30 Months													
Lung weight/BW (g/kg)	4-8	7.3	0.7	8.9	0.7	8.7	0.7	13.5[a]	1.2	6.7	0.8	11.1[a]	1.0
Lung Density (g/mL)	4-8	0.11	0.01	0.15[a]	0.01	0.14	0.01	0.29[a]	0.04	0.13	0.2	0.22[a]	0.02
Respiratory Function at 30 Months													
Total lung capacity/BW (mL/kg)	8-11	16.3	1.3	14.4	1.4	15.4	1.1	12.4	1.1	15.8	0.6	13.1	1.2
Quasistatic lung compliance (mL/cm H_2O)	8-11	0.92	0.07	0.81	0.10	0.84	0.05	0.59[a]	0.06	0.88	0.04	0.70	0.10
CO diffusing capacity/BW (mL/min/mmHg)	8-11	0.74	0.08	0.62	0.05	0.60	0.06	0.62	0.07	0.66	0.05	0.62	0.08
Forced vital capacity (FVC)/BW (mL/kg)	8-11	69	6	56	4	61	4	51[a]	4	58	4	54	3
Mean midexpiratory flow/FVC (FVC/sec)	8-11	4.9	0.6	6.0	0.6	5.4	0.4	5.8	1.0	5.0	0.4	5.1	0.5

[a] Difference from control mean significant at $p < 0.05$ by t-test and Games[5] adjustment for multiple comparisons

6.1.3 Health Effects of PAH-Enriched Emissions

Polycyclic aromatic hydrocarbons (PAH's) are present in engine exhaust because of the incomplete combustion of organic fuel during the pyrolytic process. Experimental animals have been administrated PAH's in order to determine whether or not these compounds may be held responsible for the induction of the toxic effects associated with diesel exhaust exposure. The validity of the mechanisms by which PAH's are thought to generate tumours is still the subject of debate[5,6]. But, despite the lack of consensus, the results of experiments designed to measure the tumourigenicity of these compounds is helpful in assessing the risk they represent to exposed workers.

Heinrich, Pott and Rittinghausen[39] compared the results of exposure of rodents to diesel exhaust and coal oven flue gas, which contains very high concentrations of PAH. The latter gas was used in order to determine if it was at all possible to induce lung tumours by inhalation of PAH-containing exhaust. Female Wistar rats, NMRI mice and Syrian Golden hamsters were exposed to coal oven flue gas for an average of 16 hours per day, 5 days per week for up to 22 months. A clean air exposure of as high as 12 months followed the coal gas exposure. The synopsis of the exposure periods can be found in Figure 28.

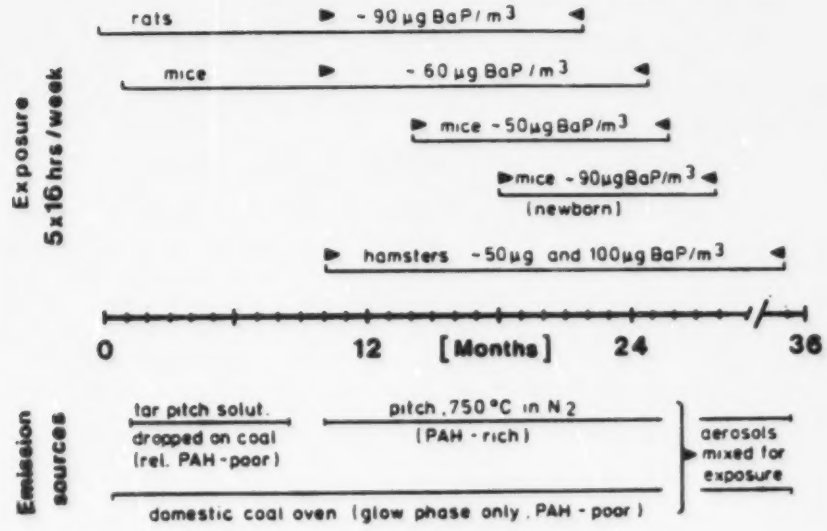


Figure 28

Synopsis of the Different Exposure Periods and BaP Concentrations of the Coal Oven/Pyrolized Pitch (COP) Exposure Atmospheres

(Source: Reference 39)

It should be noted that during the first 8 to 9 months the rats and mice received exhaust gas containing only 0.3 mg BaP/m^3.

In rats exposed for 22 months, followed by clean air exposure for 8 months, tumour incidence increased 18%, with benign tumours occurring in 16 rats and pulmonary carcinomas in 5 (Table 51).

Table 51

Lung Tumour Bearing Rats (TBR) after Exposure to Coal Oven/Pyrolized Pitch Exhaust (COP)

(Source: Reference 39)

Number of Rats with:	*Clean Air (n = 115)*	*COP Exhaust (n = 116)*
Benign Tumours	---	16
Malignant Tumours	---	5
Total number of TBR	---	21 (18.1%)

By 24 months the tumour rate in control mice was less than 1 tumour per animal. After exhaust exposure the tumour incidence and multiplicity was highly increased, even at 10 months exposure, at which time it was 86%, compared to 3.5% for control animals (Table 52).

Table 52

Lung Tumour Incidence Rate (%) and Tumour Multiplicity* in Mice After 10, 12 and 24 Months' Exposure to Coal Oven/Pyrolyzed Pitch Exhaust (COP)

(Source: Reference 39)

Months of Exposure	*Clean Air*	*COP Exhaust*
10	3.5% (0.03 ± 0.19)	85.7%** (7.9 ± 8.8)**
12	12.5% (0.2 ± 0.5)	70.0%** (3.8 ± 5.2)**
24	32.0% (0.7 ± 1.7)	79.0%** (7.0 ± 7.9)**

* Mean number ± SD of lung tumours/animal

** $p < 0.05$ compared to corresponding clean air groups by t-test (tumour multiplicity) or x^2-test (tumour incidence)

After exhaust exposure not only the incidence of adenomas increased, but there was a high percentage of pre-malignant, atypic adenomas and adenocarcinomas.

At the time of writing, the data available on the Syrian Golden Hamster was incomplete, but it was indicated that after 18 months of exposure to exhaust containing PAH at approximately 50 µg BaP/m^3, followed by clean air for 11 months, no tumours were found. However, more than 50% of the exposed animals demonstrated bronchiolo-alveolar nodular, squamous metaplasia and in 14% of the animals a papilloma was diagnosed in the larynx/tracheal region, with none being observed in clean air control animals. The data clearly demonstrated that exhaust containing sufficiently high concentrations of PAH is able to induce lung tumours in rats, mice and possibly in hamsters. It is important, however, to realize that the BaP content of the PAH-enriched exhaust was more than 1,000 times higher than found in diesel engine emissions.

The authors noted that the most prominent finding in the diesel-exposed lungs was a heavy load of soot particles, which resulted in inflammatory and fibrotic reactions. This occurred despite a higher particle mass concentration in the PAH-enriched coal oven flue gas in which the particulate concentration, at least during the last three months of the first two years of exposure, was approximately 6 mg/m^3 compared to 4 mg/m^3 for the diesel exposed animals. The latter particulate matter was primarily condensed organic material which was easily dissolved, metabolized and eliminated from the lungs and no particulate deposits occurred.

In a similarly designed experiment, Heinrich et al[52] subjected rats and mice to PAH-rich emissions from sources with comparable emission characteristics. Exposure was for an average of 16 hours/day, 5 days/week over a maximum of 22 months. Figure 29 details the exposure regimen.

There was a noticeable difference between the number of lung tumours found in animals kept in clean air and those exposed to engine exhaust (Tables 53 and 54).

The carcinogenic effect of the exhaust emissions seemed to be strongest in the mice exposed to exhaust gases from the age of 2 to 3 days onwards and for no more than 10 months (Table 54). Also, there was a significant increase in the number of lung tumours per animal when systematically or locally acting lung carcinogens were combined with PAH rich emissions. At the time this article was published an 11% lung tumour rate had been recorded and a 25% rate was expected once examination of all the animals was complete.

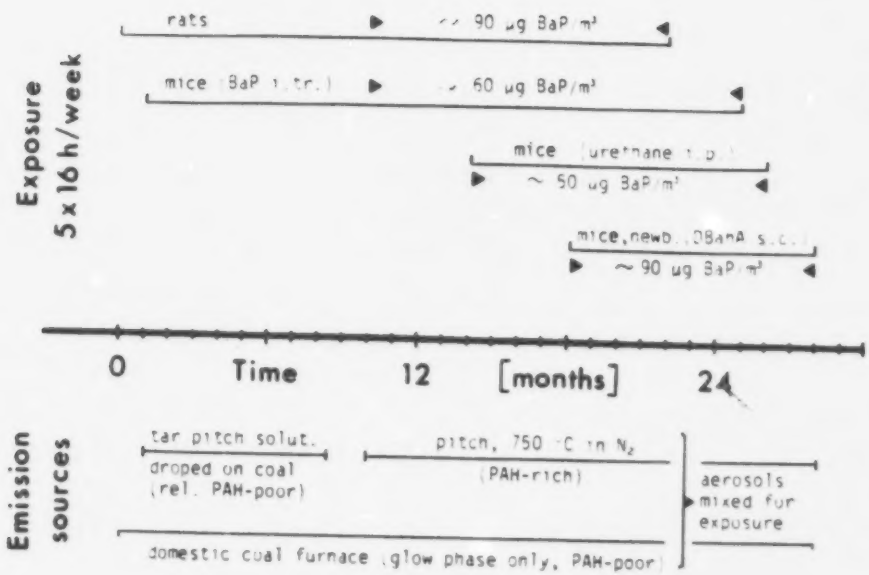


Figure 29

Synopsis of the Different Exposure Times and BaP Concentrations of the Exposure Atmospheres. Some Groups of Mice Were Additionally Treated with Carcinogens
(Source: Reference 52)

Table 53

Lung tumour Incidence[a] and Multiplicity[b] in Mice After Two Years Exhaust Exposure and Intratracheal Instillation[c] (i.tr.) of BaP
(Source: Reference 52)

Instillation i.tr.	*Clean Air*	*Exhaust*	*p-Value[d]*
Untreated (n = 28-31)	0.7 ± 1.7 32.0%	7.0 ± 7.9 79.0%	<0.001 <0.005
0.9% NaCl Sol. 20 x 30 µL (n = 55-59)	0.8 ± 1.0 54.0%	6.0 ± 6.8 81.0%	<0.001 <0.005
BaP 20 x 50 µg (n = 50-58)	2.2 ± 3.7 56.0%	8.4 ± 6.3 84.0%	<0.001 <0.005

a Percent of tumour-bearing animals
b Average number of tumours ± SD found per lung
c Once per week for 20 weeks
d Calculated by t-test (multiplicity) or x^2-test (incidence) comparing clean air and exhaust groups

Table 54

Lung tumour Incidence[a] and Multiplicity[b] in Mice Injected s.c. as Newborn Animals with DBahA and Exposed to Exhaust for 10 Months

(Source: Reference 52)

Instillation s.c.	*Clean Air*	*Exhaust*	*p-Value[c]*
0.9% NaCl Sol. (n = 26-29)	0.03 ± 0.19 3.5%	7.9 ± 8.8 85.7%	<0.001 <0.005
40 µg DBahA (n = 26-29)	3.9 ± 3.6 89.7%	19.3 ± 15.1 93.1%	<0.001 <0.7

a Percent of tumour-bearing animals
b Average number of tumours ± SD found per lung
c Calculated by t-test (multiplicity) or x^2-test (incidence) comparing clean air and exhaust groups

The carcinogenic effect of PAH-rich exhaust was clearly demonstrated after an exposure period of only ten months. Consequently, the emissions used can be classified as strongly carcinogenic.

Heinrich et al[53] investigated the carcinogenic and co-carcinogenic effects of inhaled PAH-rich emissions. In this experiment, rats were subjected to PAH-enriched coal oven exhaust for 19 hours/day, 5 days/week (to a maximum 0f 80 hours/week) for 20 months. Preliminary results revealed carcinomas in the exhaust exposed group only and not in the control group. The tumours were identified as squamous cell carcinomas and one small cell carcinoma. Also, exposed animals and controls showed little difference in clinical-chemical and hematological parameters. Lung function tests demonstrated an exposure-related decrease in compliance. However, there was no sign of alveolar lung clearance deterioration using a radioactive test aerosol.

The preliminary lung carcinoma incidence rate of 10% was considered strong evidence for diesel exhaust's cancer-inducing effect. A 25% incidence rate is expected when the study is completed.

Mohr et al[54] described the morphologic characteristics of the lungs of rats exposed to diesel exhaust and PAH-enriched coal oven flue gas and suggested a classification scheme for squamous cell tumours. This scheme has already been presented as Table 34 in a summary of two Fraunhofer Institute studies, but it is repeated here for convenience, as Table 55.

Table 55

Classification Scheme of Squamous Cell Tumours that may Occur in Exposed Rat Lungs

(Source: Reference 44)

Classification		Behaviour	Cytology
1. Benign keratinizing cystic	*Cystic*	Slow periperhal extension by air spaces	Regular, no atypia, mitoses absent or rare
2. Grade I carcinoma		Slight evidence of stromal invasion (peribronchial/ perviascular), possibly minimal scirrhous response	Borderline atypia occasional mitoses
3. Grade II carcinoma		Definite stromal invasion especially lymphatic or blood vascular, usually some scirrhous response	Mild atypia ± moderate mitotic index
4. Grade III carcinoma		Invasion/destructive/scirrhous pleural/mediastinal spread	Moderate atypia + moderate-high mitotic index
	Malignant		

Most rats from the diesel exhaust group had large pulmonary deposits of carbonaceous particles phagocytosed by alveolar macrophages, accompanied by severe chronic inflammatory changes characterized by alveolar septal thickening, bronchiolo-alveolar hyperplasia and alveolar lipoproteinosis. Squamous metaplasia occurred in 65% of the animals, with primary tumours in about 16% or 15 rats. Six rats had bronchio-alveolar adenomas, 7 had squamous cell tumours and 2 had bronchio-alveolar adenomas and squamous cell tumours. Using the classification system above, 8 of the squamous cell tumours were classified as benign, keratinizing, cystic tumours and 1 as a Grade I carcinoma. It was suggested that diesel exhaust, rather than being a complete carcinogen, might act more as a promoter of carcinogenicity resulting from severe, persistent inflammation with hyperplasia and metaplasia.

6.2 HEALTH EFFECTS OF TOTAL EXHAUST

Some of the information in this section may overlaps that in the preceeding three. Because of the all encompassing nature of their experimental design they are unsuitable for the more specialized topics covered previously. Also, their broad perspective made them appropriate for the final, comprehensive discussion that this body of work requires.

6.2.1 Review Article

Early results of a Fraunhofer Institute study[55] were reported in our previous document[1, p. 456] but will be repeated here for convenience of review. A lifetime exposure carcinogenicity/toxicity study was carried out in Syrian Golden Hamsters exposed to exhaust from a 2.4 L Daimler-Benz diesel engine at a constant load of 16 kW and 2400 rpm using fuel containing 0.36% sulphur. Running conditions were 35% load, 55% speed, equivalent to approximately an 85 km/hr cruising speed. The exhaust was split to either a mixing chamber where it was diluted 7:1 with air, or it was centrifuged to remove all the particulate matter prior to being diluted 7:1 with air. The inhalation chambers received 5 m^3 per hour of diluted exhaust with an average particulate concentration of 3.9 mg/m^3 with a median diameter of 0.1 µm. The gaseous components of the exhaust, as measured in the exposure chamber, are shown in Table 56.

Table 56

Component Concentrations of the Diluted Diesel Exhaust
(Source: Reference 55)

	In The Chambers	
	A	B
CO (ppm)	18.0 (+ 4.4)	18.5 (+ 4.9)
CO_2 (Vol %)	0.52 (+ 0.13)	0.54 (+ 0.15)
SO_2 (ppm)	2.8 (+ 1.7)	3.1 (+ 1.8)
C_NH_M (ppm)	7.9 (+ 3.3)	9.3 (+ 4.6)
CH_4 (ppm)	2.6 (+ 1.8)	3.0 (+ 2.2)
$C_NH_M - CH_4$ (ppm)	5.0 (+ 2.5)	5.9 (+ 3.0)
NO (ppm)	17.2 (+ 5.9)	16.5 (+ 5.8)
NO_X (ppm)	19.2 (+ 6.1)	18.6 (+ 5.8)
NO_2 (ppm)	1.0 (+ 1.5)	1.2 (+ 1.7)
O_2 (Vol %)	20.0 (+ 0.7)	19.5 (+ 0.6)
Particles (mg/m^3)	---	3.9 (+ 0.5)

A: Exhaust without particles; B: Total exhaust

The PAH content (µg/g) of soot was measured and is tabulated in Table 57.

Table 57

PAH Content of Soot

(Source: Reference 55)

PAH/Sample	*Diesel Exhaust*	*PAH/Sample*	*Diesel Exhaust*
FLU-	134.6	BeP-	14.1
PYR-	65.8	BaP +	7.0
BaF-	5.4	PER-	---
BbF	5.3	IP +	13.4
BaA +	9.8	DBA +	---
CHR +	25.7	BghiP-	21.4
BFL +	22.2	COR-	12.5

\- = Carcinogenic in animal experiments

\+ = Non-carcinogenic in animal experiments

Exposure was for 7 to 8 hours per day, 5 days per week for a lifetime and it was estimated that the hamsters inhaled a total of approximately 40 mg of soot during this period. The average lifetime was determined to be 80 to 82 weeks and the median experimental lifetime, at which time 50% of the animals had died, was 72 to 74 weeks (Figure 30).

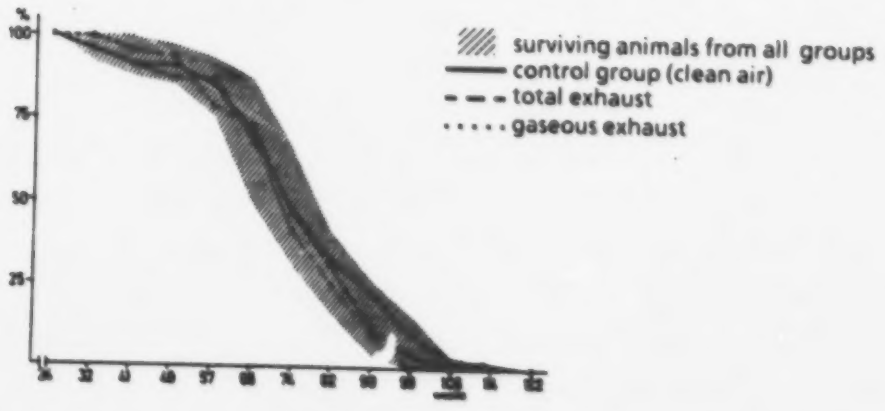


Figure 30

Surviving Hamsters at Different Times After the Start of the Experiment (in %)

(Source: Reference 55)

It was estimated that exposure to relatively diluted exhaust gas was unlikely to give statistically-significant proof of the existence or non-existence of a carcinogenic effect in the small number of animals used. for this reason, the animals were pre-treated with either dibenzo(a,h)anthracene (DB[a,h]A) or diethylnitrosamine (DEN) to increase the incidence of respiratory tract tumours.

For animals not receiving any special pre-treatment, several hematological parameters and enzyme activities were measured at the 29th, 42nd, 55th and 75th week of treatment. No consistent, significant effects could be related specifically to exhaust treatment, except as follows. After 29 weeks animals exposed to exhaust had increases in serum GOT, LDH and AP as well as gamma-GT. These results suggested that the inhaled diesel emission may have had an effect on the liver. As well, blood urea nitrogen (BUN) was elevated indicating impairment of renal function in Syrian golden hamsters exposed to diesel exhaust. Low serum creatinine levels would suggest renal damage that was not prolonged or severe. In humans, the BUN can be elevated for years and gradually rise before the serum creatinine also rises. However, in humans with elevated BUN, abnormal 24-hour urinary results show entrenched renal damage many months to several years before serum creatinine rises.

Electron microscopic examination indicated degenerative changes of the hepatocyte mitochondria. As reported previously(1,p.460), the increase in papillomas in the larynx and/or trachea of DEN-treated animals was dose-related and was significant in animals exposed to particle-free and total exhaust (Figure 31).

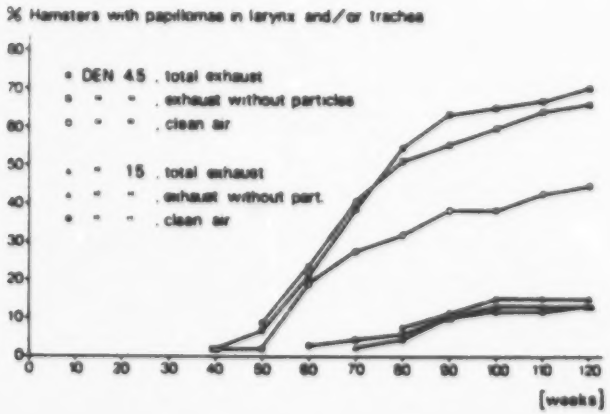


Figure 31

DEN-Treated Hamsters with Papilloma in Larynx/Trachea Expressed as a Percentage of the Animals Per Group at the Start of the Experiment. DEN = 4.5 mg/kg b.w.; DEN 1.5 = 1.5 mg/kg b.w.

(Source: Reference 55)

No significant difference in the tumour incidence among the three groups receiving different exposure atmospheres was seen and only 5 DEN-treated animals had papillomas. No animals without special carcinogenic treatment had any respiratory tract tumours, regardless of the inhalation exposure.

Discrete proliferative changes of the lung were relatively common, with about 60% being described as adenomatous proliferations. The percentage of hamsters exhibiting this response was particularly high in the groups receiving intratracheal installations, but the highest incidence of proliferative growth was consistently seen in animals exposed to total exhaust emissions (Figure 32).

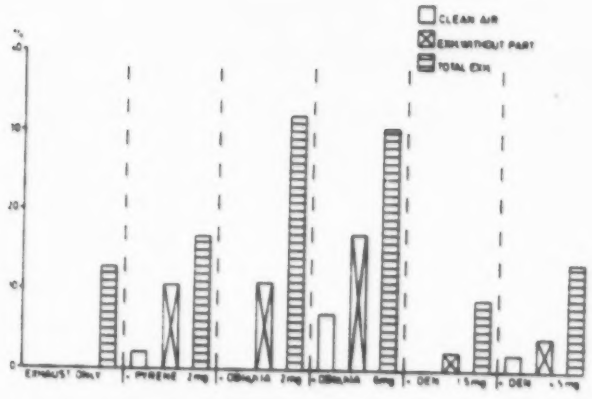


Figure 32

Percent of Hamsters with Focal Proliferations in the Lung. There are 5 Treatment Groups (Pyren,DB[a,h]A,DEN) and 1 Group Without Additional Pretreatment

(Source: Reference 55)

In a parallel study, female Wistar rats were exposed for up to 24 months to the same experimental exhaust conditions as the hamsters. Lung clearance rates were measured after 18 months of exposure using hematite iron oxide aerosol ($^{59}Fe_2O_3$). Long-term alveolar clearance was impaired in the total exhaust exposed rats and, to a lesser extent, in those exposed to the gaseous exhaust phase (Figures 33 and 34).

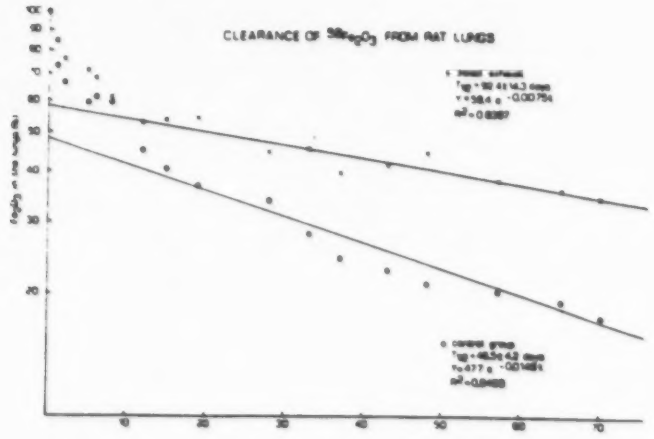


Figure 33

Lung Clearance of $^{59}Fe_2O_3$-Particles in Rats Exposed to Total Diesel Exhaust
(Source: Reference 55)

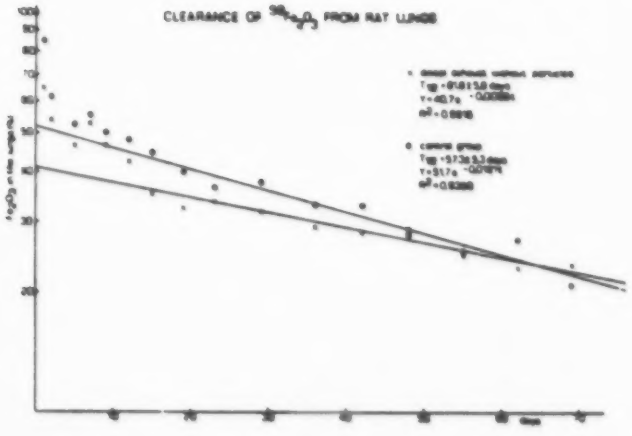


Figure 34

Lung clearance of $^{59}Fe_2O_3$-Particles in Rats Exposed to Total Diesel Exhaust Without Particles
(Source: Reference 55)

The half-life for the pulmonary elimination of the iron oxide deposit in animals exposed to non-particulate components of exhaust was about 40%

longer than for control animals. For those animals exposed to total exhaust the half-life was nearly twice as long as for controls. Thus, exposure to total exhaust emission lowered the bronchial clearance.

Various pulmonary function tests were carried out on rats after two years of exposure to exhaust with or without particulate. As can be seen in Table 58 no significant changes in respiratory rate, minute volume, compliance or resistance were measured.

In a more recent abstract(56), the same group reported that in rats exposed to diluted diesel exhaust containing approximately 4 mg/m^3 of particulate for 90 hours per week, lung tumours were detected in 13 out of 95 exposed rats compared to 0 out of 95 control animals. As well, mice exposed to similar conditions demonstrated a significantly increased incidence of lung carcinoma. Presumably the rat data were obtained from the same group of animals as the pulmonary function information discussed above. The abstract also quoted that no lung tumours were found in diesel-exposed hamsters, which presumably refers to the hamster study detailed previously.

Table 58

Pulmonary Function Test of Anesthetized Rats After Two Years Exposure With Total Diesel Exhaust or Gaseous Exhaust Without Particles

(Source: Reference 55)

	Control	*Gaseous Exhaust*	*Total Exhaust*
Respiratory Rate (breath/min.)	49.6 ± 12.2 (50.6 ± 12.2)	47.6 ± 22.6 (44.9 ± 21.2)	57.5 ± 18.0 (56.4 ± 17.8)
Tidal volume (mL)	2.6 ± 0.51 (2.6 ± 0.43)	2.9 ± 0.61 (3.1 ± 0.90)	2.67 ± 0.64 (2.5 ± 0.52)
Minute volume (mL)	127.5 ± 32.1 (132.0 ± 22.5)	138.3 ± 74.5 (131.2 ± 52.8)	147.5 ± 39.5 (137.9 ± 40.7)
Intrapleural pressure (cm H_20)	4.0 ± 0.77 (4.7 ± 0.92)	4.6 ± 1.1 (6.1 ± 2.0)	4.0 ± 0.97 (4.1 ± 1.010)
Compliance (mL/cm H_20)	0.82 ± 0.25 (0.71 ± 0.14)	0.79 ± 0.15 (0.82 ± 0.31)	0.84 ± 0.27 (0.78 ± 0.26)
Resistance (cm M, Ω/mL•sec^{-1})	0.14 ± 0.03 (0.16 ± 0.03)	0.19 ± 0.16 (0.25 ± 0.18)	0.14 ± 0 05 (0.17 ± 0.09)
Breathing time quotient (tT exp./T insp.)	0.53 ± 0.14 (0.58 ± 0.19)	0.53 ± 0.15 (0.72 ± 0.25)	0.60 ± 0.13 (0.56 ± 0.16)
Heart rate (pulse/min.)	317 ± 41.5 (323 ± 37.7)	334 ± 43.5 (324 ± 42.00	305 ± 32.9 (309 ± 33.5)

With acetylcholine inhalation ( ); Acetylcholine concentration = 0.3%; Exposure time about 1/2 minute

6.2.2 Exposure to Total Exhaust Coupled with Co-Carcinogen Pretreatment

A few researchers have chosen to administer a co-carcinogen at the same time as they exposed their subjects to diesel engine exhaust. The reason for the use of a co-carcinogen is the same as that given in those cases where high exhaust concentrations are chosen: it is to compensate for the relatively small number of animals that must necessarily be used in bioassays. Since the tumour incidence for exposure to diesel engine exhaust alone is very low, the added effect of the co-carcinogen maximizes the chances of seeing a positive toxic response.

Takemoto et al[57] from the Saitama Medical School in Japan conducted a long term inhalation study using a small diesel engine. They tried to elucidate the tumourigenic effect of diesel exhaust in rats and mice. Female F344/Jcl rats, C57BL/6N mice and newborn ICR mice were exposed to diesel exhaust generated using a small Yanmar 269cc diesel engine run at an idle of 1600 rpm. Air was diluted 1:4 before entering an inhalation chamber in which the animals were exposed for 4 hours per day, 4 days per week for 2 years.

Rats were assigned to four experimental groups, as follows:

Group A - diesel exposed
Group B - di-isopropanol-nitrosamine (DIPN)
Group C - diesel plus DIPN
Group D - control

Rats in groups B and C were given IP injections of DIPN (1 g/kg BW) once a week for three weeks from one month after the start of inhalation. In the case of mice no carcinogen was used, with half the animals being exposed to diesel exhaust and half receiving only dilution air.

The average concentration of pollutants in the inhalation chamber were CO, 40-100 ppm; NO, 30-6 ppm; NO_2, 2-4 ppm; SO_2, less than 0.5 ppm; particulate, 2-4 mg/m^3, with a mean diameter of 0.32 µm. PAH concentrations in the inhaled exhaust were measured and contained B(a)P, 0.85 µg/g; B(ghi)P, 5.9 µg/g and B(k)F 7.8 µg/g. 1-NP was present at a concentration of 93 µg/g.

At fixed intervals throughout the 24 month study period, animals were randomly selected from the exposure groups for autopsy. Histopathology of the lung alveoli and terminal bronchioles showed only hyperplasia until tumours showed near the end of the experiment with F344 rats. The results appear in Table 59.

Table 59

Lung Tumour Caused by Diesel Exhaust Exposure in F344 Rats with DIPN

(Source: Reference 57)

Group	6 Months				12 - 17 Months				18 - 24 Months			
	No.	Ah.	Ad.	Ca.	No.	Ah.	Ad.	Ca.	No.	Ah.	Ad.	Ca.
A: Diesel	4	0	0	0	7	0	0	0	15	2	0	0
B: DIPN	3	0	0	0	8	4	2	0	21	15	10	4
C: Diesel + DIPN	5	0	0	0	18	15	12	3	18	15	12	7
D: Control	3	0	0	0	5	0	0	0	12	0	0	0

No.: Number of rats; Ah.: Alveolar hyperplasia; Ad.: Adenoma; Ca.: Carcinoma;
DINP: 1 g/kg (b.w.) i.p., weekly x 3

Two animals exposed to diesel exhaust demonstrated alveolar hyperplasia after 18 to 24 months of exposure, but no adenomas or carcinomas were observed. As expected, the use of pre-treatment with di-isopropanol-nitrosamine (DIPN) resulted in an increase in the frequency of both alveolar hyperplasia, adenomas and carcinomas from 12 months to the end of the experimental period. Exposure of DIPN treated rats to diesel exhaust significantly increased the number of animals with alveolar hyperplasia after 12 to 17 months of exposure, as well as increasing the incidence of adenomas and carcinomas from 12 months through to termination at 24 months.

Similar data for ICR mice can be found in Table 60.

Table 60

Lung Tumour Caused by Diesel Exhaust Inhalation in ICR Mice (Newborn)

(Source: Reference 57)

Group	Sex	3 - 6 Months (No.)	7 - 12 Months			13 - 18 Months			19 - 28 Months		
			No.	Ah.	Ad. - Ca.	No.	Ah.	Ad. - Ca.	No.	Ah.	Ad. - Ca.
Diesel Expos.	M	42	29	0	0	15	2	1	11	3	2
	F	22	20	0	0	15	2	0	11	3	1
Control	M	24	19	0	0	19	2	0	10	1	0
	F	21	17	0	0	19	1	0	12	2	1

No.: Number of mice; Ad.: Adenoma; Ad. - Ca.: Adenoarcinoma

Although adenomas and adenocarcinomas were observed after 13 to 18 months of treatment, the differences in the malignant tumours between the diesel exposed and non-exposed groups was not statistically significant.

For C57BL mice the incidence of lung tumours is shown in Table 61.

Table 61

Lung Tumour Caused by Diesel Exhaust Inhalation in C57BL Mice (Newborn)

(Source: Reference 57)

Group	Sex	3 - 6 Months (No.)	7 - 12 Months			13 - 18 Months			19 - 28 Months		
			No.	Ah.	Ad. - Ca.	No.	Ah.	Ad. - Ca.	No.	Ah.	Ad. - Ca.
Diesel Expos.	M	25	28	0	0	40	2	1	33	4	2
	F	13	10	0	0	39	2	1	38	4	1
Control	M	6	4	0	0	7	0	0	17	1	0
	F	6	4	0	0	12	0	0	15	0	0

No.: Number of mice; Ad.: Adenoma; Ad. - Ca.: Adenoarcinoma

In this instance there appears to be a significant increase in the incidence of adenomas and adenocarcinomas in diesel exposed animals from 13 months of treatment. In fact, adenocarcinomas were only observed in diesel exposed mice.

Inflammatory lesions were frequently found in the lungs of diesel exposed rats and mice, which was more prominant than in control animals. Although slight, chronic inflammatory lesions were found in the nasal cavity of control animals, in treated animals the inflammation had progressed significantly with exudation into the nasal cavity being common.

Although in the rat studies no evidence of diesel related lung tumour formation was noted, there were changes such as pneumonia, thickening of the alveolar walls, emphysema and hyperplastic lesions. In rats, in which the carcinogen DIPN was administered, the addition of diesel exhaust resulted in an additive incidence of tumours, suggesting that diesel exhaust may have had a significant role in the development of lung tumours.

In the mouse studies two strains of mice with very low spontaneous tumour incidences were used. The data demonstrate a trend, but no increase in tumour induction as a result of diesel exhaust exposure.

In a study done by the American Environmental Protection Agency (EPA)[58] a Nissan 6 cylinder diesel engine was used to generate exhaust to which Sencar mice were exposed 8 hours per day for 7 days a week for 15 months. Test groups included clean air control mice exposed to diesel exhaust who had either been administered butylated hydroxytoluene (BHT) as a tumour initiator weekly for 13 weeks, or a promoting or co- carcinogen single dose of urethane and animals untreated, but exposed to diesel atmosphere.

Exhaust dilution was adjusted to provide a diesel suspended particulate level of 6 mg/m^3 for the first 19 weeks of exposure and 12 mg/m^3 for the duration of the study. Table 62 gives a summary of the principal pollutants in the clean air supply.

Table 62

Concentration of Principal Pollutants in the Clean Air Supply

(Source: Reference 58)

Component	*Average Concentration ± SEM (Weeks 1 - 124)*
Carbon dioxide, %	0.039 ± 0.002
Carbon monoxide, ppm	2.20 ± 0.50
Total hydrocarbons, ppm C	2.82 ± 0.50
Nitric oxide, ppm	0.051 ± 0.039
Nitrogen dioxide, ppm	0.03 ± 0.027
Sulphur dioxide, ppm	0.032 ± 0.023

Several groups of animals were used including the following: A) BHT intraperitoneally (i.p.) (included because it had been shown that BHT induced type I alveolar damage followed by Type II cell proliferation and that multiple doses augmented urethane-induced pulmonary adenomas); B) a single i.p. injection of urethane at 6 weeks of age, which was expected to act as a promoting or co-carcinogenic agent. Group C were included to test for the induction of tumours in mice receiving only diesel exhaust exposure, with no initiation or promoting agent being used. Positive control mice were intended to demonstrate susceptibility to urethane and BHT. In addition, saline and corn oil vehicle control groups were included and a group of negative control mice, which received no injections at all, were also studied. Overall pooled data showed a 13% lower survival rate for diesel exhaust exposed mice than for other groups (Figure 35)[58].

In general the data suggest at least some degree of diesel exhaust associated suppression of survival. As well, a consistent suppression of body weight gain in diesel exhaust animals was noted.

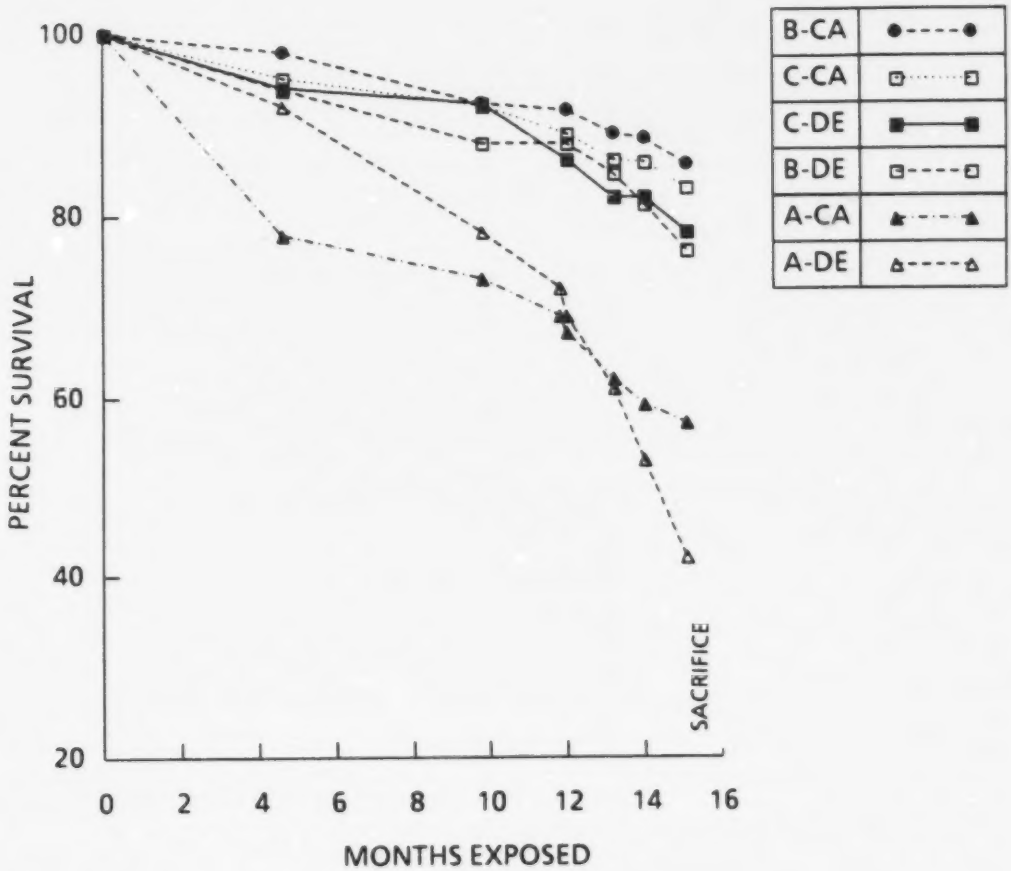


Figure 35

Diesel Oncogenicity Study in Sencar Mice - Survival
(Modified from Reference 58)

Lung tumours in surviving mice are tabulated in Table 63[58]. Although there was a trend suggesting an increase in the incidence of tumours in diesel exposed animals, none of the values achieved statistical significance. The authors indicated however that the incidence and/or degree of severity of pulmonary lesions was consistently greater in diesel exhaust exposed mice of both sexes than in clean air animals. The non-neoplastic pathology reported indicate marked inflammatory changes such as alveolar interstitial thickening, perivascular and peribronchial mononuclear cell infiltration, alveolar macrophages, black alveolar pigment and cholesterol clefts as well as black pigment in the mediastinal lymph nodes.

Table 63

Lung Tumours[a] in All Surviving Mice Examined

(Source: Reference 58)

Atmosphere:	*CA (Control)*			*DE (Test)*			*Clean (Room) Air*		
Subgroup:	*A*	*B*	*C*	*A*	*B*	*C*	*Positive Controls*	*Negative Controls*	*Saline Controls*
Tumour Sex									
Alveolar bronchiolar adenoma (ABA) M	7/82 (8.5)	10/109 (9.2)	[illegible]	[illegible]/34 (8.8)	8/89 (9.0)	4/101 (4.0)	7/12 (58.3)	0/24 (0)	6/72 (8.3)
F	11/66 (16.7)	8/114 (7.0)	7/11 (6.3)	3/77 (3.9)	9/107 (8.4)	17/104 (16.3)	8/9 (88.9)	3/29 (10.3)	
M + F	18/148 (12.2)	18/223 (8/1)	11/216 (5.1)	6/111 (5.4)	17/196 (8.7)	21/205 (10.2)	15/21 (71.4)	3/53 (5.7)	
Alveolar bronchiolar carcinoma (ABC) M	0/82 (0)	0/109 (0)	0/105 (0)	1/34 (2.9)	1/89 (1.1)	2/101 (2.0)	5/12 (41.7)	0/24 (0)	2/72 (2.8)
F	1/66 (1.5)	2/114 (1.8)	1/111 (0.9)	2/77 (2.6)	4/107 (3.7)	0/104 (0)	4/9 (44.4)	0/29 (0)	
M + F	1/48 (0.7)	2/223 (0.9)	1/216 (0.5)	3/111 (2.7)	5/196 (2.6)	2/205 (1.0)	9/21 (42.9)	0/53 (0)	
Total (ABA + ABC) M	7/82 (8.5)	10/109 (9.2)	4/105 (3.8)	4/34 (11.8)	9/89 (10.1)				
F	12/66 (18.2)	10/114 (8.8)	8/111 (7.2)	5/77 (6.5)	17/104 (16.3)				
M + F	19/148 (12.8)	20/223 (9.0)	12/216 (5.6)	19/111 (8.1)	19/205 (9.3)				
Pooled Totals	51/587 (8.7)			50/512 (9.8)					

[a] Data entries are: No. with tumour(s)/No. examined; and ( ) - % with tumour(s)

6.2.3 Exposure to Total Exhaust Without Pretreatment

The Lovelace Institute in Albuquerque, New Mexico have been involved in evaluating the long term carcinogenicity of diesel exhaust emissions in rodents for some time. Results of their studies have been made available in a series of annual reports in 1980[59], 1982[42], 1983[60], 1984[61] and 1985[62] which were summarized in a recently published paper[63]. The findings were shown to be reproducible[64].

Fischer-344 rats and CD-1 mice were housed in whole body exposure chambers and treated with either clean air or diluted diesel exhaust from a 1980, 5.7 L Oldsmobile diesel engine containing particulate and gaseous emissions as summarized in Table 64.

Table 64

Concentrations of Key Components of Exposure Atmospheres (Mean [SE] of Weekly Mean Values During 30 Months of Exposure)
(Source: Reference 63)

Component	*Units*	*Control*	*Low*	*Medium*	*High*
Soot	$\mu g/m^3$	10 (10)	350 (70)	3470 (450)	7080 (810)
Carbon monoxide	ppm	1 (1)	3 (1)	17 (7)	30 (13)
Nitric oxide	ppm	0	0.6 (0.3)	5.4 (1.5)	10.0 (2.6)
Nitrogen dioxide	ppm	0	0.1 (0.1)	0.3 (0.2)	0.7 (0.5)
Hydrocarbon vapor	ppm	3 (1)	4 (1)	9 (5)	13 (8)
Carbon dioxide	%	0.2 (0.04)	0.2 (0.03)	0.4 (0.06)	0.7 (0.1)

The exposure was for 7 hours per day, 5 days per week from young adulthood for the remainder of their life span, which was for up to 24 months for mice and 30 months for rats.

The effects of exhaust inhalation on the long term clearance using ^{134}Cesium particles in rats after 30 months of exposure is shown in Table 65.

At the two highest particulate exposure conditions the animals demonstrated significant decreases in their ability to clear particulate matter. Particle accumulation was associated with lung inflammation cell proliferation, epithelial metaplasia and fibrosis. In lung and lung-associated lymph nodes there was a diffuse increase in particle-laden macrophages within alveolar spaces, focal collection of such macrophages adjacent to terminal bronchioles with alveolar

wall fibrosis, bronchiolar metaplasia and, rarely, squamous metaplasia. Lung-associated lymph nodes were consistently enlarged and contained densely packed clusters of particle-containing histocytes in both the peripheral sinusoids and within the cortex. Findings in medium level rats were similar, but less severe and foci of alveolar wall thickening were less common. In rats receiving the lowest dose of diesel particulate only occasional clusters of macrophages containing phagocytosed particles were observed.

Table 65

Effects of Exhaust Inhalation on the Long-Term Clearance Component of ^{134}Cesium Particles in Rats after 30 Months of Exposure
(Source: Reference 60)

Exposure Group	*Long-Term Component (Percentage of Total Clearance)*	*Half-time of Particle Clearance*[a]
High	26	686 ± 79[b]
Medium	24	748 ± 82[b]
Low	25	204 ± 40
Control	22	197 ± 16

[a] Mean ± standard deviation;
[b] Significantly different from control mean at $p<0.001$ by F test

There were no consistent indications that exposure to diesel exhaust altered body weights of animals or life expectancy(61), indicating a low degree of general toxicity of diesel exhaust, even under exposure conditions causing significant lung disease. Examination of rat lungs for evidence of carcinogenicity were reported in References 62 and 63. Following 24 months of exposure the accumulated amount of soot increased progressively in the lungs of rats at all exposure levels being 20.8, 11.5 and 0.6 mg in the high, medium and low exposure groups, respectively.

Four types of lung tumours were found and those seen for each treatment group are shown, by type and time of observation, in Figure 36(63).

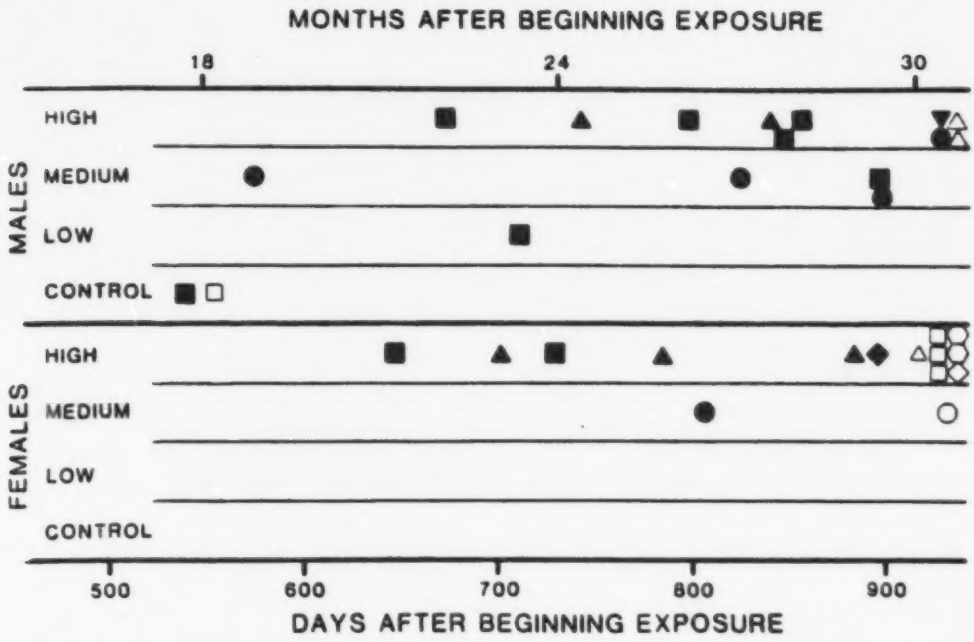


Figure 36

Type and Time of Lung Tumours Observed
Tumour type indicated by symbols: ○ = adenoma, □ = adenocarcinoma; △ = squamous cyst; ▽ = squamous cell carcinoma; ⬡ = two adenocarcinomas and ◇ = one adenocarcinoma and one squamous cyst. Filled symbols indicated rats dying or euthanized and open symbols indicate rats sacrified.
(Source: Reference 63)

Tumours appeared to be derived from epithelial cells and consisted of benign and malignant forms of two primary types. Bronchio-alveolar adenomas were typically small and cells of these benign tumours did not invade adjacent structures. Adenocarcinomas often resembled the adenomas at their periphery, but had central areas with hyperchromatic cells of non-uniform size arranged in a solid, irregular pattern. Increased mitosis and invasion of adjacent vessels or interstitial connective tissue were observed. Squamous cysts were structures composed of keratin cores surrounded by well-differentiated, uniform squamous epithelial cells. None of the lung tumours were found to have metastasized to pulmonary lymph nodes or other organs.

A total of 36 tumours were found in 32 of 553 rats examined. Four females in the high dose group had two tumours each; two had two adenocarcinomas and two had one adenocarcinoma and one squamous cyst. The remaining tumour-bearing rats had one tumour each. Only adenocarcinomas were found in the

control and low groups and only in male rats. A tumour incidence of about 1% was observed in control rats receiving the low dose of diesel particulate, which is consistent with the incidence found for untreated F344 rats. The percentages of rats with tumours, among those examined for tumours, were 16.1%, 4.6% 0.7% and 1.4% in the high, medium, low and control groups, respectively (Table 66).

Table 66

Percentages of Rats with Lung Tumours
(Source: Reference 63)

Group	*Adenomas*	*Adenocarcinomas + Squamous Cell Carcinomas*	*Squamous Cysts Only*	*All Tumours*
High	0.7	9.8*	5.6*	16.1*
Medium	3.8*	0.8	0	4.6
Low	0	0.7	0	0.7
Control	0	1.4	0	1.4

*Significantly higher than control at $p<0.05$, by z statistic

The findings were consistent with an initiation-promotion mechanism of carcinogenesis. Additional evidence came from the fact that mutagenic and carcinogenic species are present in diesel soot. This was supported by the finding that DNA extracted from lungs of high level exposed rats had increased numbers of adducts, in comparison to DNA from control animals.

It is suggested that the interaction of soot-associated organic compounds with lung cell DNA might be the initiating event. Chronic inflammation and epithelial cell proliferation could have acted to promote tumour formation. There was a clear dose-response relationship between the occurrence of lung tumours and exhaust exposure, which was not linear, but progressive.

The Automobile Research Institute of Japan sponsored a long-term inhalation study in rats to examine the effects of diesel exhaust from heavy and light duty diesel engines[65]. A light duty 1.8 L IDI diesel engine, at 1/3 load and 1700 rpm (50 kph) and a heavy duty (HD) 11 L, 6 cylinder, DI engine run at 1/2 load and 1200 rpm (40 kph) were employed. The LD exhaust was diluted 10-15:1 and the HD exhaust 13-15:1. Animals were exposed 16 hours per day, six days per week for 30 months. Table 67[65] gives an outline of the study design and conditions.

Table 67

Outline of Long-Term Inhalation Studies on the Effect of Diesel Emissions

(Source: Reference 65)

Series:	*Heavy Duty*										*Light Duty*				
Experiment:	• *Carcinogenicity* • *Long-Term Inhalation*					• *Particle Influence*					• *Carcinogenicity* • *Long-Term Inhalation*				
Group (mg/m³):	4	2	1	0.4	0	4	4*	0.4	0.4*	0	2	1	0.4	0.1	0
Number of Rats: (each group):	M - 120; F - 95					M - 64					M - 120; F - 95				
Engine Speed (rpm)	1,200 (40 km/h)										1,700 (50 km/h)				
Torque (kg•m)	35.4 (max. 74.0 kgm/1,400 rpm)										4 (max. 11.2 kgm/2,000 rpm)				
Fuel Consumption (g/min)	170 ± 1										39.3 ± 0.4				
Dilution Ratio	13~15										10~15				

Table 68 provides a summary of the gaseous and particulate concentrations to which the animals were exposed during the 30 month experimental period. Average quantities of B(a)P in the diluted exhaust from the LD and HD series was 4.4 and 2.8 µg/g, respectively. Values for 1-nitropyrene were 57.1 for the LD and 15.3 µg/g for the HD exhaust. No significant differences were observed in hematological or clinical chemistry findings between the experimental and control groups during the first 24 months. Diesel particulate was deposited in the interstitial tissue and penetrated into the alveolar walls, as well as being present in the regional and mediastinal lymph nodes. The incidence of lung tumours in the various groups is given in Table 69.

The changes in the airway surfaces of the trachea, through to the respiratory bronchioles and alveoli were evaluated using electron microscopic techniques. In animals receiving particulate exhaust the main changes in the airways, prior to 30 months, were irregularity, shortening and loss of cilia from the ciliated epithelia and irregularly arranged protrusions or hypotrophic foci in the non-ciliated epithelia. Epithelial hyperplasia was classified into two grades. Grade 1 was mild, focal, scattered and reactive and Grade II was moderate, diffuse, confluent hyperplasia with occasional papillary projections, stratifications and slight cellular atypia, without nodular formation.

Table 68

Summary of Gaseous and Particle Concentrations (30-Month Inhalation)

(Source: Reference 65)

Experiment	*Group (mg/m^3)*	*Particle Concentration (mg/m^3)*	*Gaseous Concentration*										
			NO_X (ppm)	*NO (ppm)*	*NO_2 (ppm)*	*CO (ppm)*	*CO_2 (%)*	*SO_2 (ppm)*	*HTHC (ppmc)*	*LTHC (ppmc)*	*HCHO (ppm)*	*SO_4^{2-} (µg/m3)*	*O_2 (%)*
Carcino-	4	3.72	37.45	34.45	3.00	12.91	0.360	4.57	15.65	7.62	0.29	361	20.4
genicity &	2	1.84	21.67	19.99	1.68	7.75	0.215	2.82	9.94	5.90	0.18	198	20.6
Long-term	1	0.96	13.13	12.11	1.02	4.85	0.140	1.79	7.15	5.16	0.11	111	20.7
Inhalation	0.4	0.46	6.17	5.71	0.46	2.65	0.084	0.98	4.63	4.27	0.05	62.9	20.8
(HD)	0	0.002	0.061	0.042	0.021	0.63	0.035	0.06	2.43	3.50	0.003	0.49	20.8
Particle	4	2.99	36.45	31.50	4.95	12.90	0.412	4.03	13.79	7.68	0.20	358	20.3
Influence	4 (Free)	0.019	36.76	32.81	3.96	13.00	0.391	4.50	12.68	7.79	0.24	1.61	20.4
(HD)	0.4	0.39	5.81	5.37	0.44	2.50	0.084	0.98	4.53	4.41	0.04	57.7	20.7
	0.4 (Free)	0.005	5.58	5.16	0.42	2.54	0.083	0.96	3.74	4.43	0.04	1.43	20.7
	0	0.004	0.062	0.040	0.024	0.06	0.068	0.03	2.38	3.62	0.003	0.35	20.8
Carcino-	2	2.32	20.34	18.93	1.41	7.10	0.418	4.70	5.49	3.57	0.13	315	20.3
genicity &	1	1.08	10.14	9.44	0.70	3.96	0.219	2.42	3.82	2.87	0.07	151	20.5
Long-Term	0.4	0.41	4.06	3.81	0.26	2.12	0.105	1.06	2.93	2.51	0.03	62.4	20.7
Inhalation	0.1	0.11	1.24	1.16	0.08	1.23	0.050	0.38	2.44	2.27	0.01	18.8	20.8
(LD)	0	0.003	0.044	0.033	0.011	0.80	0.026	0.06	2.20	2.17	0.002	0.41	20.8

Table 69

Incidence of Lung Tumour

(Source: Reference 65)

	Group	Sex	No. of Rats	Adenoma	Carcinoma	Incidence in Males and Females (%)	Incidence in the Group (%)
LD Series	2 mg/m³	M	64	0	2	3.1	
		F	60	1	0	1.7	2.4
	1 mg/m³	M	64	0	3	4.7	
		F	59	0	2	3.4	4.1
	0.4 mg/m³	M	64	1	0	1.6	
		F	61	0	0	0	0.8
	0.1 mg/m³	M	64	0	1	1.6	
		F	59	1	1	3.4	2.4
	0 mg/m³	M	64	0	2	3.1	
		F	59	1	1	3.4	3.3
HD Series	4 mg/m³	M	64	0	5	7.8	
		F	60	0	3	5.0	6.5
	2 mg/m³	M	64	0	3	4.7	
		F	59	0	1	1.7	3.3
	1 mg/m³	M	64	0	0	0	
		F	61	0	0	0	0
	0.4 mg/m³	M	64	0	1	1.6	
		F	59	0	0	0	0.8
	0 mg/m³	M	64	0	0	0	
		F	59	0	1	1.7	0.8

After inhalation for 30 months of light duty diesel exhaust Grade II hyperplasia was observed as follows: 2 mg/m³ group, 70.2%; 1 mg/m³, 9.8%; 0.4 mg/m³, and less than 0.05% for the 0.1 and 0 mg/m³ groups. Following heavy duty diesel exposure the 4 mg/m³ group had incidences of 20.2%, the 2 mg/m³, 11.4% and less than 6% in the 1.4 and 0 mg/m³ groups. The incidences of hyperplasia after 30 months were higher than the corresponding values after 24 months. The incidence of leukemia and breast tumours in the experimental animals after 30 months was not significantly higher than for the control groups.

The incidence of lung adenomas and carcinomas is summarized in Figure 37, which demonstrated that a significant difference occurred between the 4 mg /m3 group and 0 mg/m3 groups of the heavy duty exhaust groups.

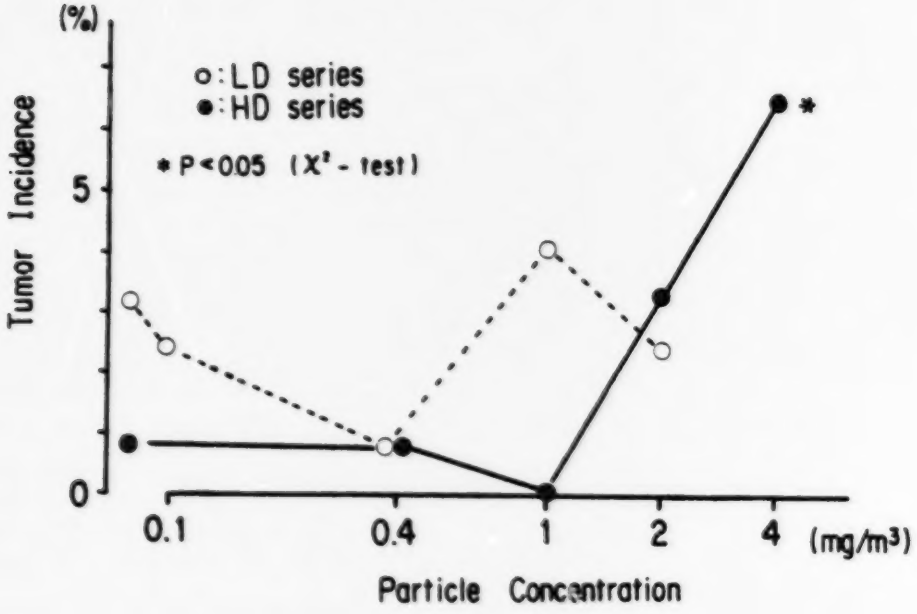


Figure 37

Relation Between Incidence of Lung Tumour and Particle Concentration
(Source: Reference 65)

Experimental conditions and preliminary results of General Motors studies into the health effects of diesel exhaust were discussed in the last report[1] (also see reference 332 from this same report or Reference 66 from the present edition). In this study three nominal concentrations of diesel particulate of 250, 750 and 1500 µg/m3[66] were used. Exhaust was generated using a 1978 5.7 litre IDI Oldsmobile diesel engine run at a steady speed of 1350 rpm and a constant load of 96 Nm, which simulated 65 km/hr cruising speed. Exposure conditions for the GM experiments were presented in Reference 332 from the 1984 publication[1] (or Reference 66 of this report) and are repeated here for convenience (Table 70).

Table 70

Inhalation Chamber Environmental Parameters[a]

(Source: Reference 66)

	Control	*1500 µg/m-3*	*750 µg/m-3*	*250 µg/m-3*
Chamber temperature (°C)	21.5 ± 0.7	21.8 ± 1.0	21.6 ± 0.7	21.6 ± 1.1
Relative humidity (%)	48 ± 9	51 ± 9	48 ± 7	44 ± 10
Dilution ratio (approx.)	---	40:1	80:1	240:1
Nitrogen oxides (mg/m-3)	0.05	9.2 ± 1.6	5.0 ± 1.2	2.1 ± 0.6
Carbon monoxide (mg/m-3)	2.2 ± 0.6	7.9 ± 2.1	5.3 ± 0.9	3.4 ± 0.8
Carbon monoxide (mg/m-3) (corrected for ambient)	0	5.7	3.1	1.2
Aerosol concentration (µg/m-3)	7 ± 9	1533 ± 346	796 ± 228	258 ± 87[b]

[a] Mean ± standard deviation over all measurements between 12/8/78 and 12/19/80

[b] 261 ± 178 including one filter of 8042 µg/m-3 drawn during high exposure on 12/18/79

Preliminary information was made available by GM which was included in the 1984 up-date and which indicated an increased incidence of tumour formation with chronic exposure(1). Subsequently, additional data has been made available(6,67). These studies were conducted at the Southwest Research Institute on behalf of General Motors, and were considered significant as the particulate levels in the two lower exposure groups were similar to those found in underground dieselized mines(1). A/J strain of mice were reported as not responding with any increase in tumour incidence to any level of exhaust exposure. Similarly, meaningful tumour data were not obtained in the Hartley hamster, but in Fischer 344 rats an increase in pulmonary tumours was observed(6,67).

Out of 90 animals exposed for 15 months followed by an 8 month recovery period of exposure to clean air, an incidence of 5 bronchio-alveolar carcinomas was observed. No carcinomas occurred in any of the 30 control animals who received clean air for 23 months, nor were any observed in the 180 animals exposed to diesel exhaust for 9 or 15 months without a recovery period, or in 60 control animals associated with these test periods. One tumour occurred in each of the 250 and 1500 mg/m3 diesel particulate groups and 3 in the 750 mg/m3 group, and therefore, there appeared to be no dose-response relationship. Experimental and control animals had evidence of focal pneumonia and it was suggested that this might have been a confounding factor.

7. WHOLE ANIMAL, NON-INHALATION STUDIES

The results of short duration, non-inhalational experiments offers only limited information compared to longitudinal studies with exposure by inhalation. The details of these limitations will be discussed in the appropriate section. Despite the fact that extrapolations from them are restricted, they allow the identification of those exhaust fractions that are carcinogenic to mammals. Also, they may shed light on the mechanism of toxicity. For these reasons, the results have been included.

Deutsch-Wenzel and Brune[68], in their analysis of coal oven flue gas, determined that PAH's consisting of 4 to 6 rings and azaarenes exhibit a comparable carcinogenic potency. However, the effect of the latter group would be mainly due to PAH's with 6 or more rings since azaarenes have been shown to contribute only 4 to 7% of the total activity. According to a raw estimate, no more than 2 to 3% of the total carcinogenic activity of flue gas condensate would be attributable to benzo(a)pyrene.

The mutagenicity of nitropyrenes has been discussed. Their potential carcino-genicity remains the greatest source of concern for both exposed individuals and for health officials. Investigations of these compounds' toxicities were designed in an effort to determine the extent to which nitropyrenes represent a measurable threat.

Sato et al[69] intratracheally instilled Syrian golden hamsters with 0.5 mg of 1.6-dinitropyrene (1.6-DNP) in 0.2 mL of saline weekly for 26 weeks. The results, which are quite unmistakable, are shown in Table 71.

Table 71

Induction of Tumours in Syrian golden Hamsters by Intratracheal Instillation of 1,6-Dinitropyrene
(Source: Reference 69)

Group	*Initial Number of Hamsters*	*Effective No. of Hamsters*	*Tumours*	
			Adenocarcinoma of the Lung	*Leukemia*
1,6-DNP (0.5 mg)	M - 10 F - 10	10 10	10 9	6 6
Control	M - 10 F - 10	10 10	0 0	0 0

In oral intubation or intraperitoneal injection studies[50], rats were subjected to 1-nitropyrene (1-DNP) and 1,3-DNP, 1,6-DNP and 1,8-DNP in dimethylsulphoxide (DMSO) or to DMSO only (for controls). Ten micromoles of the compound were administered per kilogram of body weight, three times each week for four weeks. The total cumulative dose was 16 µg per rat. The observed tumour rates upon sacrifice, if the animals were moribund, or after 76-78 weeks were as shown (Figures 38 and 39).

Figure 38

Development of Mammary Tumours Following Intraperitoneal Injection of Dimethylsulfoxide (DMSO), 1-nitropyrene (1-NP) or 1,3- 1,6- or 1,8-dinitropyrene into Weanling Female CD Rats

(Source: Reference 70)

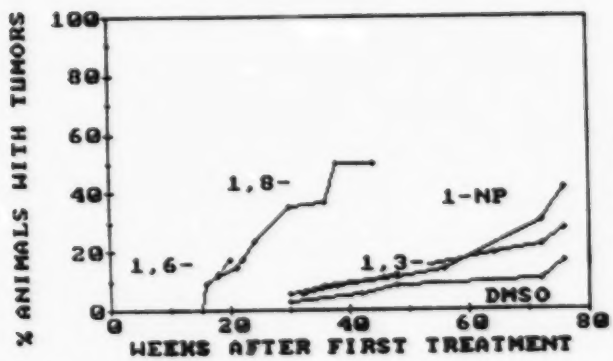


Figure 39

Development of Mammary Tumours Following Oral Intubation of Dimethylsulfoxide (DMSO), 1-nitropyrene (1-NF) or 1,3-, 1,6- or 1,8-dinitropyrene into Weanling Female CD Rats

(Source: Reference 70)

The majority of mammary tumours observed after intraperitoneal injection of pyrene derivatives were adenocarcinomas. Animals that survived more than 21 weeks all developed multiple soft tissue tumours in the peritoneal cavity. The latter tumours were classified histologically as malignant fibrous histiocytomas (MFH). Control animals developed only benign fibroadenomas. The mammary tumours that appeared after oral intubation were primarily benign fibroadenomas.

In a similar investigation, rats were injected subcutaneously with the same compounds as above. They were treated eight times at weekly intervals for a total dose of 6.3 µg of compound. Tumourigenic response was determined as above in moribund rats, or in animals that were sacrificed at the end of 67 weeks. The results appear in Figures 40 and 41.

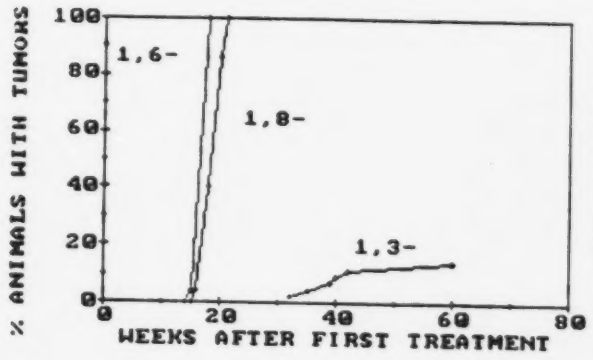


Figure 40

Development of Malignant Fibrous Histiocytomas Following Subcutaneous Injection of Dimethylsulfoxide (DMSO), 1-nitropyrene (1-NP) or 1,3-, 1,6- or 1,8-dinitropyrene into Newborn Female CD Rats

(Source: Reference 70)

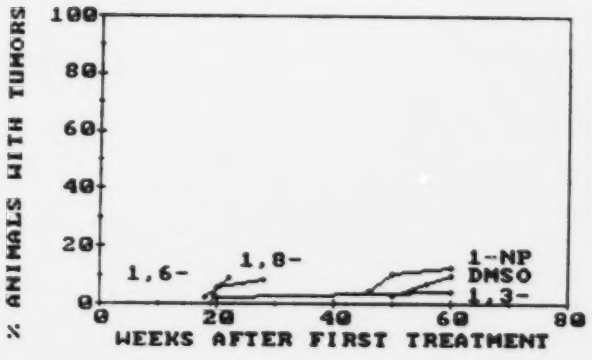


Figure 41

Development of Mammary Tumours Following Subcutaneous Injection of Dimethylsulfoxide (DMSO), 1-nitropyrene (1-NF) or 1,3-, 1,6- or 1,8-dinitropyrene into Newborn Female CD Rats

(Source: Reference 70)

1,6- and 1,8-DNP produced MFH's rapidly at the injection site. 1.3-DNP induced these malignant tumours in only 14% of the rats. Mammary tumours were observed in a low percentage of the animals less than 20 weeks old. The tumours detected early were malignant adenocarcinomas, whereas those that appeared later were primarily fibroadenomas.

The investigators concluded that, in these experiments, the induction of malignant fibrous histiocytomas following intraperitoneal or subcutaneous injection clearly demonstrated the potent carcinogenicities of 1,6- and 1,8-DNP. The other two nitropyrenes appeared less carcinogenic. The absence of MFH or mammary tumours after oral administration of the compounds may be due to inadequate absorption, or, alternately, to metabolism in the gastrointestinal tract.

Kawabata et al[34] reported on a study of the carcinogenicity of diesel particles, activated carbon, and solvent instilled in the lungs of rats. Groups of animals were subjected to the following treatments: 1) 10 mg of diesel particles (1 mg/week for 10 weeks) administered intratracheally IT; 2) 10 mg of activated carbon (1 mg/week for 10 weeks) instilled IT; 3) 2 mL of solvent (0.05% Tween 80 in phosphate buffered saline, 0.2 mg/week for 10 weeks) given IT.

The tumour incidences are listed in Table 72.

Table 72

Incidence of Lung Epithelial Tumours After Instillation of Solvent, Activated Carbon and Diesel Particles
(Source: Reference 34)

	Control	*Solvent*	*Activated Carbon*	*Diesel Particles*
Number of Animals	50	23	23	42
Lung Tumours	0	1	11 +	31* (37**)
Benign Tumours	0	0	4	11
Malignant Tumours	0	1	7 +	20 (26*)

\+ $P<0.01$ between control or solvent and activated carbon
* $P<0.05$ between activated carbon and diesel particle
** $P<0.01$ between activated carbon and diesel particle

The researchers felt that the high incidence of tumours observed in the activated carbon group was most surprising. They concluded that this material's physical and biological action on the epithelium resulted in damage that initiated the development of hyperplastic and ultimately, neoplastic changes in the lungs. Hyperplasia of the non-ciliated epithelium in terminal bronchioles and of the alveolar epithelium was cited as evidence in support of the theory.

8. EPIDEMIOLOGY

Epidemiology has rarely been of much help in finding a relationship between diesel engine exhaust and cancer. Even though epidemiologists may have to re-evaluate their analytical approach, their results have been included for consideration.

Sorlie et al[71] used the data of the Framingham study to assess the influence of cigarette smoking on lung function. The cohort included 2,336 men and 2,873 women that ranged in age from 30 to 62 years. It was found that the follow-up forced expiratory volume (FEV) was lower in cigarette smokers and that the members of this group displayed poorer lung function performance than non-smokers after 14 years. Also, smokers weighed less on average and had higher hematocrit values than non- smokers. On the other hand, those who quit smoking have better lung functions on follow-ups.

Since cigarette smoking usually is considered more hazardous than exposure to diesel exhaust (because PAH concentrations are higher in the former emission)[14], less serious health effects can probably be expected from diesel emissions.

Wynder and Higgins[72] reviewed a number of epidemiological studies that examined the role of diesel engine emissions in the development of lung and bladder cancer. They concluded that, although truckers, bus drivers and others with potential occupational exposure to diesel engine exhaust may be at increased risk of bladder cancer, there is insufficient evidence to ascertain that the increased risk is due to diesel exposure or to occupation. Similarly, their results were encouraging in that they provided little evidence of lung cancer risk from diesel exhaust emissions.

In two earlier epidemiological reviews[73,74] by the same authors, the same conclusions had been reached: *"diesel exhaust exposure was not considered a serious health hazard"*[74].

A 35-year study[75,76,77] of mortality in Ontario INCO (International Nickel Company) workers examined a large cohort, extensive data and a long time period: it included 54,509 men with six months or more service during the 35 year period that started on January 1, 1950 and ended at the end of 1984.

It was concluded that lung cancer excess at one of the nickel refining plants could be explained entirely by exposure to high, airborne nickle oxide/sub-sulphide areas. In another case, miner's lung cancer mortality was tentatively attributed to copper/nickel ore dust exposure. More generally, Roberts et al[76] observed that the two high risk areas corresponded to the dustiest areas at which nickel was concentrated. Clearly, airborne particulate matter represents a tangible risk, irrespective of its composition.

Also, an unexpected pocket of lung cancer mortality in lead welders and cranemen is of some relevance. Cancer and death in the former group was explained by a potential

exposure to PAH's: welders were required to heat a coal tar based mastic sealant in repair operations. Cranemen primarily work in travelling cranes mounted in the roof of the tankhouse. Because the roof is a natural collecting point for electrolyte vapours and other fumes, these workers are at increased risk.

Rantanen[88] examined the relationship between lung cancer incidence and occupational exposure to potentially toxic inhalable substances in Finnish dieselized underground mines. The author reported on studies that found Finnish miners have an increased risk of lung cancer. The SMR of miners was 2.08, compared with 1.0 for the entire economically-active population (Table 73). Several types of risks concentrate within the population of miners, including high mortality from cancer and accidents. The exhaust products of diesel engines, which came into use in the 1950's and the early 1960's, may contribute to the observed lung cancer incidence. To shed some light on this possibility, the polycyclic aromatic hydrocarbons (PAH's) of the ambient air were studied. The total concentration of particulate varied between 0.9 and 27 mg/m^3 and the total concentration of PAH's ranged between 181 and 801 ng/m^3. Given these values, the possible role of exposure to diesel engine exhaust fumes in the elevated risk to lung cancer of miners is being investigated in Finnish mines.

Table 73

Incidence of Lung Cancer in Men Aged 35 - 69 in Mining and Quarrying[a]

(Source: Reference 88)

	Lung Cancer Incidence per 10^5		
	Observed	*Expectred*	*SMR*
Whole branch	42	20.2	2.08b
Mining	27	13.0	2.08b
Deep drilling	2	1.4	1.42
Concentration	4	1.4	2.92
Other mining and quarrrying	9	4.4	2.03

a Data of Pukkala et al (Sosiaalikaaket. Aikak. Lehti 6:363, 1979)

b Statistically significant at level $p \leq 0.001$

Garshick et al (1987a)[89] conducted a case-control study of deaths among U.S. railroad workers to test the hypothesis that lung cancer is associated with exposure to diesel exhaust. The study included only male railroad workers who had at least 10 years of railroad service, were vested in in the railroad retirement program, were born on or after January 1, 1900, and died between March 1, 1981 and February 28, 1982. Death certificates for 87% of the 15,059 deaths reported to the U.S. Railroad Retirement Board were collected.

Within the cohort, 1,256 workers who died from lung cancer were matched with two deceased comparison workers by age (±2.5 years) and date of death (±31 days). Deceased workers whose jobs had involved exposure to diesel exhaust (engineers and firemen, brakemen and conductors, diesel locomotive repair workers and hostlers) were compared with deceased workers without occupational exposure to diesel exhaust (clerks and station agents). Workers exposed occupationally to diesel exhaust for 20 years had a significantly increased relative odds ratio (1.41, 95% confidence interval = 1.06 - 1.88) of lung cancer.

In another study by Garshick et al (1988)[90], U.S. railroad workers aged 40 to 64 in 1959 who started railroad service 10 to 20 years earlier were included. The observation period started in 1959 and ended in 1980 and included deaths that occurred before December 31, 1980. Only 88% of death certificates were collected. The data showed that workers aged 40 - 44 in 1959 had a significantly increased relative risk (1.45, 95% confidence interval = 1.11 - 1.89) of lung cancer.

Data on cigarette smoking were collected in a case control study (Garshick et al, 1987b)[91]. An attempt was made to control for the confounding exposure to asbestos. Attempts were also made to characterize exposures to diesel exhaust through the collection of industrial hygiene data. The period between the first diesel exposure and data analysis was adequate to allow the observation of exposure-related cancer for some age groups of the cohort. The fact that the findings of the two studies were both independent (the two studies based their analyses on different lung cancer deaths) and consistent, fortifies the conclusion that occupational exposure to diesel exhaust is associated with an increased risk of lung cancer.

Albert and Chen[78] presented an interesting comparison of the relative carcinogenicity of diesel exhaust, cigarette smoke, roofing tar and coke oven exposure. For the latter three, carcinogenicity data are available from epidemiological studies (relative to life-time risk) for humans Table 74.

Table 74

Unit Human Lung Cancer Risks from Exposures to Coke Oven Emissions, Roofing Tar and Cigarette Smoke
(Source: Reference 78)

Exposures	*Unit Risk* (*lifetime risk/µg organics/m³*)
Coke Oven Emissions	9.3×10^{-4}
Roofing Tar	3.6×10^{-4}
Cigarette Smoke	2.2×10^{-6}

Using *in vivo* and *in vitro* determined measurements, the authors estimated lung cancer risk exposed to the particulates emitted by various engines. They calculated that the unit risk from Nissan, Volkswagen and Oldsmobile small diesel engines is one order of magnitude greater than risk from a Caterpillar heavy duty diesel engine (Table 75).

Table 75

Unit Lung Cancer Risks Estimates for Various Diesel Exhaust Particulates
(Source: Reference 78)

Emission Source	*Unit Risk Estimates (lifetime cancer risk/μg/m³)*
• Nissan	4×10^{-5}
• Volkswagen Rabbit	2×10^{-5}
• Oldsmobile	2×10^{-5}
•Caterpillar	2×10^{-6}

Using data from the EPA sponsored Lovelace rat carcinogenicity study, the authors concluded that the predicted potency of the diesel engine particulate was consistent with the results of the inhalation study, as well as with the results of studies in which mouse skin tumour initiation tests were performed. They concluded that the results supports "the utility of the comparative mouse skin tumour potency method for risk assessment of organic combustion products and the validity of the rat lung as an indicator of human response to this class of carcinogens".

Tominaga[112] from the Aichi Cancer Centre Research Institute in Japan provides another source of practical comparisons.

> *"If we take a look at B(a)P concentrations, I understand diesel exhaust emission contains approximately 50 ng/m³, and if we assume the gas is diluted by a factor of 100 (maybe it is in fact diluted by a factor of 1000), and if we inhale such diluted exhaust emissions for 50 years, and assuming we inhale 20 m³ of air a day, then we inhale approximately 10 ng of B(a)P/m³, which is equivalent to smoking 0.6 cigarettes a day because from other studies we know that on the average, one cigarette yields 16.1 ng of B(a)P. So if we use B(a)P as an index, then inhaling diluted diesel exhaust emissions is approximately equivalent to smoking 0.6 cigarettes a day.*
>
> *If we take the mutagenicity based on the Ames Test (in this case I used the data of TA98 system with S9 mix) from Dr. Onishi's experiment, diesel exhaust gas yields 27,400 revertants/m³. If the gas is diluted by a factor of 100, and if we inhale 20 m³ of air a day, it gives us 5,480 revertants a day. From a study by Dr. Ogaki and Dr. Sato of the National Cancer Center, we know that one cigarette yields about 13,700 revertants so that 5,480 revertants is the equivalent of 0.4 cigarettes. Thus, if we use B(a)P, we end up with an equivalent of 0.6 cigarettes, and if we take a look at mutagenicity we end up with 0.4 cigarettes a day.*
>
> *In this way, we obtain some rough ideas to what extent diluted diesel exhaust has health effects on humans. That is a simple calculation. This is one way to extrapolate the health effects of experimental studies."*

9. LIMITATIONS OF RESEARCH

The presence of irritants, mutagens and co-carcinogens in diesel engine exhaust does not automatically imply that these emissions represent a serious health hazard. For instance, Muir and Verma[79] commented: "*The evidence suggests that the underground polycyclic aromatic hydrocarbons, PAH's are undesirable and, that efforts should be made to reduce them but that the health impact as far as lung cancer is concerned is likely to be small.*" Similarly, Albert[80]noted: "*the unequivocal demonstration of excess lung cancer from exposure to diesel engine exhaust particles is not very likely in human populations.*"

After the presentation of so many experiments with negative results, comments like those cited above may seem to belittle the potential risk that diesel engine emissions represent. However, one should realize that vigilance is in order when attempting to interpret or to extrapolate from the results of relevant investigations. Indeed, several people have written on the fact that the data from many of the reports on the toxicity and carcinogenicity of diesel emissions may be of limited value.

The most frequently expressed concern is that, often, "*in the rodent carcinogenesis bioassay of a chemical, an excess incidence of tumours is observed only after the prolonged administration of high dose levels of the test substance.*"[5] (emphasis added). Some have argued that such exposure conditions are necessary if the possible toxic effects are to appear in the relatively small number of animals used in these studies[80,81]. However, according to Stott and Watanabe[5], it is unlikely that these data can serve in any meaningful extrapolation of the risks to humans unless interspecies pharmacokinetic comparisons and information regarding the resultant macromolecular events are also gathered.

Current research is slowly solidifying the base of knowledge related to the second of the two restrictions imposed above. However, interspecies pharmacokinetic comparisons are plagued by problems of their own as discussed below.

Looking at more specific problems, Vostal suggested that the estimates made from the results of mutagenicity tests in bacteria may be exaggerated. This would mean that the corresponding potential for toxicity may never achieve in humans the extent it has reached in microorganisms.

The degree of interspecies variability in response has been significant even among rodents. For instance, Stober[43] from the Fraunhofer Institute concluded that exposure to diesel exhaust allowed to establish a causal relationship between soot deposition in

the lung and the induction of lung tumours only in the case of rats. Vostal[82] reached the same conclusion after reviewing of the results of a number of experiments.

Because of these findings, it is conceivable that rats may be more sensitive to diesel exhaust than other experimental animals. This possibility is reassuring in that the risk to humans may not be as high as first expected. In fact, Vostal[82] does not see a need to be overly concerned with the likelihood of a dramatic increase in lung cancer in populations exposed to the emissions. However, the absence of consistent tumour incidence data among the different test species makes difficult the task of interpreting and estimating accurately the risk that diesel emissions represent.

It should be noted that, when selecting data for extrapolation, the EPA usually chooses those from the most sensitive species tested. Perera[83] supports this procedure. She backed this position with evidence by Crouch and Wilson and also the Safe Drinking Water Committee:" *This is not only consistent with the aim of being health protective, but is based on evidence that humans are, in most cases, as sensitive as or more sensitive than experimental animals.*"[83]

It is difficult to extrapolate results obtained from animal experiments to humans. Indeed, McClellan and Perera[83] do not recognize extrapolation of health effects from experimental data or risk estimation as the major goal of animal investigations. Perera[83] wrote: ". . .*great difficulties arise in extrapolating from generally limited pharmacokinetic studies in experimental animals to humans whose activation, detoxification and repair systems may be substantially influenced by other host and environmental factors not operating in the test species.*" For these reasons, the information from whole animal and *in vitro* studies is considered valuable for understanding the underlying mechanisms of toxicity, mainly.

Continuing with the limitations of diesel emission investigations: Heinrich et al[39] from the Fraunhofer Institute have acknowledged the possibility of producing tumours of the respiratory tract or of other organs by the epicutaneous, subcutaneous, intratracheal or intrapulmonary (or, certainly, intraperitoneal) administration either of selected pure PAH's or of organic material extracted from the particulate matter contained in exhaust. However, the toxic, and even carcinogenic manifestations observed in these non-inhalation tests, may not be representative of the risk that lies in exposure to diesel exhaust. This is because the direct administration of these compounds eliminates the need for their dissolution from the particles to which they are firmly bound under field conditions. Also, the absence of the irritant gases or particles eliminates the contribution of these fractions to the overall health effect of the emissions.

Consequently, a possible synergism, or simple additivity of exhaust components' effects is lost.

Heinrich et al[81] made pertinent comments on the doses used in different investigations. Although chemical compounds have been shown to be carcinogenic on their own, they are so diluted in exhaust that tumourigenic effects cannot be expected. In the future, they suggested, individual unmeasured toxic materials or combinations of numerous substances, each beneath their maximum allowable concentration, should be tested for more meaningful data.

Perera[83] considers epidemiological studies to be of limited usefulness in hazard evaluation and risk assessment of potential human carcinogens, because of their insensitivity and the lack of reliable exposure data from which to derive dose-response relationships. Tominaga[14] listed some of the problems associated with epidemiological studies. To be considered is the influence of confounding factors such as diet, occupational exposure and, more importantly cigarette smoking. Also, an adequate latency period of maybe 10 to 15 years, should be allowed. Equally important is the exact quantitation of exposure concentrations and, related to this, the need for accurate dosimetry. Misfeld and Abel[87] added the lack of distinction between histologic types of lung cancer cases as additional limitations that plague the data. Measures must be taken to correct the above mentioned problems. *"Hopefully, in the future, there will be fewer opportunities for successful epidemiological studies because control strategies will have limited the exposure of people to toxic agents"*[86].

Finally, Kondo[84] emphasized that the etiology of cancer is still unknown. As a consequence, the possible causes of tumour initiation and the resultant symptoms remain empirically related, at best. Unfortunately, the lack of the linking information impedes the elaboration of an accurate and dependable risk assessment scale.

Kondo's comments hinted to the fact that, in the present situation where the health effects of diesel engine emissions are being evaluated, only positive results are conclusive. Whereas a false negative may occur as a result of low exposure dosage, a false positive would be improbable, not to say impossible, given what is known of the mechanisms likely to be responsible for the observed toxicity and tumourigenicity.

10. HUMAN HEALTH IMPLICATIONS

by: C. Anne Mildon, M.D.
Prepared with the assistance of Lidia Kuleshnyk, B.A.

In this addendum regarding diesel exhaust emissions used in whole animal tissue and cell research, there has been a major emphasis on mutagenicity, tumour development and carcinogenicity. Ethically, animals have had to be used but the problems of extrapolating the animal-generated material to human health experience have been remarked on many times in the scientific world. These problems were alluded to many times in the 1984 edition of our examination of diesel emission health effects(1).

Nevertheless, thanks to animal studies, computer models and some recent studies on human groups exposed to occupational diesel exhaust, our knowledge of the health effects of diesel exhaust emissions, their effects on humans as well as greater detailed knowledge of lung tumours and cancers has grown in the past few years.

Consequently, it would be informative to compare what has been learned from the most recent animal studies with what is known about human lung cancer, cell changes and health effects. There are also reviews of several recent studies on large human groups exposed to diesel exhaust emissions.

10.1 ANATOMICAL PATHOLOGY OF TUMOURS AND CANCERS

Unfortunately, the lay public has come to equate "tumour" with "cancer". Tumours, however, are space-occupying lesions (usually due to an overgrowth of cells) which can be benign or malignant in which case the tumour is called a cancer.

10.1.1 Locations of Lung Tumours and Cancers

10.1.1.1 Human Pathology

In the earlier editions of our diesel exhaust review(1) the locus of cancers was still considered to be in the mid-central region, usually in the bronchus within three generations of the main bronchi division.

Actually, tumours can arise in any part of the lung, but autopsy and surgical studies had shown central growth were 45 to 54% of all lung cancers, depending on the study.

However, more careful review of x-rays, surgical specimens and autopsy studies showed that in the past 20 years approximately 60% of all lung cancers originated in the periphery of lungs. Later studies showed that 60% of the cancers arose distal to a segmental bronchus or more

distal than the third generation of dividing bronchi. Furthermore, about 20% of the lung cancers were in the far periphery of the lung and even more interesting was the finding that these peripheral cancers arose in relation to an area of scarring or fibrosis[92, 93].

Saccomano et al[94] in their intensive 20-year study of smoking uranium miners on the Colorado plateau, noted that metaplasia and tumour developed at the "spur" of dividing bronchi. Tissue review, studies with animal lungs both in the chest and outside as laboratory specimens as well as computer models have shown that the spur of dividing bronchi is the locations where most carcinogens from the exterior impinge. This is true in both turbulent air flow in larger bronchi and in laminar air flow in small airways 2 mm and smaller in diameter. Eddies in air flow form around these spurs and particles and gas molecules impinge on the spurs where metaplasia is developing.

10.1.1.2 Metaplasia

Squamous metaplasia, as so frequently referred to in the reviewed animal studies and in human pathology and cytology[92, 93, 94], is frequently found in the lining of airways where chronic prolonged inflammation has been going on. Instead of the tall rectangular epithelial cells, the lining cells broaden, flatten, lose their cilia and therefore malfunction in the lungs' and airways' natural clearing mechanisms. Squamous metaplasia is seen most extensively in smokers but in smaller areas in patients with chronic bronchitis and in people living in highly-polluted environments[92]. Because findings are found more in populations where bronchogenic cancer arises, studies continue to see if this squamous metaplasia is a pre-cancerous lesion[92, 93, 94].

Demonstration in animals of diesel particular uptake in distal bronchiolar (non-ciliated) epithelium was demonstrated by Kawabata et al[34].

Further, Spencer et al[92] have reviewed multiple human pathology studies and pointed out that there is an increased incidence of cancer in the right lung over the left lung and that most studies shown more lung tumours of all kinds in the upper lobes especially in the right upper lobe of humans.

In a more recent study, Groth et al[95] found a significant difference in deposition and retention of aerosols between animal lungs

and human lungs. Animal studies have indicated aerosol deposition is largely in the apex region of the lung whereas the studies of Groth et al have shown predominant deposition of aerosol particulate in the mid-lung region in humans.

This may pose a problem in extrapolating some data from animal studies to human experience. However, these studies will require repetition and clarification.

10.1.1.3 Animal Pathology

At present, the pathological studies done as part of animal inhalation studies which are reviewed in this edition bear out the human findings of Spencer et al and Saccomano.

The studies as reported by the Fraunhofer Institut, Battelle-Geneva and some of the Japanese institutes do not clearly indicate the exact lung zone in which tumours and cancers are found[43, 44, 45, 46]. However, cross-references indicate both localized and confluent tumourous changes. Several studies[34, 41, 61, 62, 65] indicate the relation of tumours especially cancers as arising near scar tissue.

Ishinishi et al[65] gave a clear description of tumours arising not only in association with fibrosis (scar tissue), but also in the subpleural region of the lungs, similar to those found in humans[92, 93].

10.2 CELL TYPES IN HUMAN LUNG TUMOURS AND CANCERS

10.2.1 Cellular Types in Humans

The most recent autopsy and surgical specimen studies show squamous cell cancers are found in humans 38 to 56% of the time. Untreated, this cancer seems to lead to the longest life expectancy. But even though there are excellent results after surgical removal, re-growth can occur several years later[92, 93]. In humans, cancers can spread by the lymph or blood system but in the animal studies, these distant cancer relocations called metastases were not seen[64]. At present, the lack of metastases in lab animals has not been explained. The metastases seen in humans frequently spread to vital organs and are usually the main cause of human pain and are often the major cause of death.

Studies of human pathology have shown the occurrence of adenocarcinoma including scar cancers range from 11 to 28% of human lung cancers. The latest studies show that there has been a steady increase in the incidence of adenocarcinoma.

10.2.2 Review of Animal Tumours

Reviews of animal studies incorporated in this current update mention squamous cell and adenocarcinoma the most frequently[39, 43-46, 57, 63]. Bronchioloalveolar carcinoma[43, 63] is also seen in these animals, a lung cancer seen in humans with an incidence of 4 to 5% of all lung cancers. This lung cancer too is considered to be increasing in incidence and some pathologists consider the incidence to be closer to 10%[92].

One cancer mentioned in only one of the animal pathology studies[53] reviewed here is small cell (for example oat cell carcinoma), which is found in humans as 37% of all lung cancers[92]. Furthermore, this has in the past thirty years been the most malignant of the human carcinomas and death occurs usually 4 to 24 months after diagnosis. The tumours are usually small and difficult to spot especially on chest x-rays. While still in the human these cancers give rise to metabolic and endocrine disturbances. They also metastasize easily and quickly[92].

Of greatest importance is the fact that from 1964 to 1986, Saccomano, Archer and other researchers who were studying the uranium miners on the Colorado plateau found that 79% of the lung cancers in these miners were small cell lung cancers. Two of the co-carcinogens in the epidemiology of these cancers were radioactive radon daughters and cigarette smoking[94, 96 - 98].

Eventually, we can expect scientists to develop an animal model of oat cell lung cancer that develops with exposure to the pollutants that miners (especially uranium miners) are exposed to, but at present the animal cancers (predominantly rat cancers) in the diesel exhaust studies include cancers seen in humans which are squamous cell, adenocarcinoma and bronchioloalveolar carcinoma. Unfortunately, a major lung cancer seen in humans which is oat cell cancer has not shown in the major animal studies reviewed in this update, which makes comparison of animal and human data more difficult. Further studies with an oat cell model of cancer in animals are needed.

10.3 HYPOTHESES OF CELLULAR DEVELOPMENT TO MALIGNANT TUMOURS

10.3.1 Squamous Cell Lung Cancer Development

The lining of the airways is made up of tall columnar epithelium. When squamous cell cancers in the airways are examined, the main cancer cell is a squarish cell with atypia as features of the nucleus of the cell. Review of cells at the edge of the cancer shows squamous cell metaplasia and, as mentioned earlier,

these nests of metaplastic cells develop at the spurs of dividing bronchi or bronchioles[41, 54, 92, 94].

As time progresses over 10 to 20 years with exposure to carcinogens and other contributing factors, atypia increases in the metaplastic cells until they become typified as dysplastic, usually designated as mild, moderate or marked. Eventually the tumour becomes carcinoma *in situ* and finally invasive carcinoma ensues[93, 94]. After invasion, the carcinoma spread to other surrounding tissue. then in this process cancer cells are carried in the blood stream and lymph fluid to distant locat. ns where tumourlets there are called metastases[92].

10.3.2 Metastases

In our report[1, pg. 103] it was pointed out that none of the lung tumours were observed to have metastasized (or spread to a distant site) such as the lymph nodes around the lungs of the animals or to other organs. This was emphasized in the Lovelace Institute research on rats and mice[60, 62-63].

However, in the natural course of disease, when malignant tumours occur in humans, the cancers invade lymph nodes and nearby organs by 3extension into surrounding tissue. Also cancer cells spread by the lymph system to more distant tissue and also spread by the blood stream to distant organs. In fact, no other cancer spread so widely as lung cancer[92]. Most frequently distant sites were secondary cancers or metastases are found are liver, adrenals, brain, skeleton, kidneys and spleen[92].

Furthermore, cancers from other sites in the human body frequently implant in the lungs as metastases. For example, kidney, prostate, testicular, bone, lymph node cancer and leukemia often develop secondaries in the lungs.

Surgery to remove the primary cancer in the lung has been implicated in some studies in causing the cancer cells to shower into the blood stream and spread to distant sites[92]. However, other factors such as toxic pollutants have been dealt with in a vague manner or have been only rarely studied until recently.

One study by Richters et al examined the effect of mice (C57BL/6J) inhaling nitrogen dioxide (NO_2) on the fate of circulating cancer cells and the development of metastases in lung tissue with an increase in mortality rate[99]. The cells were not lung cancer cells but were melanoma cells injected intravenously into the mice. The injections were undertaken after 12 weeks of exposure to NO_2 at the level of 0.4 ppm while control animals were exposed to filtered air.

Twenty-one days after the melanoma cell injections, the control mice had 31 cancerous nodules in their lungs, whereas the NO_2-exposed mice had 83

melanoma nodules in their lungs. Autopsies showed the increased mortality in subpopulations of the exposed mice was due to lung metastasis progression and then involvement of the lungs, thoracic cavity and mediastinum.

The metastases are one of the major complications that inhibits medical professionals in successfully treating humans with any cancer and even more so with lung cancer. Since increased mortality was seen in the earliest days (days 29-36) following development of the mice lung nodules of melanoma, it is concluded that the mortality rate shows disease progression in the mice who demonstrated greatest sensitivity to NO_2. Such sensitivities to radiation and variations in biological response have been recorded before among inbred mice(99).

The authors postulate that ambient level of noxious air pollutants may have some impact on the progression of cancer to mortality and the effect of these pollutants may be greater than previously thought(99).

Extrapolating the above study to human experience we must point out that human beings for various reasons are subject to a number of cancers. This is true both in workers above the ground as well as in miners in the underground environment. We can leave aside the question of whether diesel exhaust emissions causes cancers in animals and possibly in humans because this question has been so well addressed in the Fraunhofer(43,44), Battelle-Geneva(45), Lovelace Institute(60, 62, 63) and Vostal(6,82) studies, as well as so many other studies reviewed herein. We must ask whether humans, especially miners, exposed to ambient or near-ambient levels of diesel exhaust or fractions of diesel emissions (as in NO_2) are going to suffer worsening of cancers they are heir to due to genetic background or lifestyles. Also are these miners going to have increased metastases and mortality due to these noxious pollutants which they were exposed before developing their cancers. The Richters et al study and the few others that barely expand on the subject cannot clarify if exposure to pollutants from childhood or in adult life worsen cancer later on. However, the Richters study presents a different and new view on the progression of cancer. This study opens the door to many more questions regarding cancer morbidity and mortality and indicates urgently-needed studies on the role of pollutants at ambient levels in influencing cancers such as bowel or skin cancer as well as lung cancer which so many humans would acquire in the course of their life(99).

10.3.3 Multiple Factors Leading to Cancer

There has been some debate amongst scientists regarding the relative importance of the system whereby fractions of diesel emissions with mutagenic activity (such as PAH's) causes atypia in the DNA in the nucleus of the bronchiolar

and alveolar epithelial cell, an effect which is called genetic because the mutagenic activity affects the genome. The other important system has been labelled epigenetic because the DNA or genome was not affected directly but rather there was a toxic effect by pollutants or an overload of particulate in the lungs, often inert dust or soot in the respirable range, leading to scarring (fibrosis) and later associated with tumour and/or cancer. The arguments and animal studies have been well-reviewed in pages 3 - 4 and 71 - 86 of Reference 6.

10.3.3.1 Fractions of Exhaust and Health Effects

A) Non-Mutagenic Fraction - Inert Soot

It has been recognized fro some time that overloading lungs with particulate even soot leads to uptake by lung and alveolar macrophages (AM) and then if the AM becomes overloaded, the clearance system can break down.

(i) Alveolar Macrophage Clearance

P.E. Morrow[100] reviewed a number of animal and human cell (AM) studies and developed some measurements which showed lung clearance breakdown. He found that the clearance breakdown was due to loss of mobility of the AM when it becomes too overloaded with particles. It became evident that when the particulate volume exceeds 60 μm^3/AM in the Fischer 344 rat, the overload effect begins. When the distributed particulate volume exceeds about 600 μm^3 per AM cell, the clearance mechanism comes to a halt and the overloaded AM remains in the alveolar space. In fact, scanning electron microscopy has shown these cells cannot put out pseudopods to move across surfaces to reach the mucociliary system and leave the lungs.

The overloaded AM remaining in the lungs has toxic effects. Neutrophils (related to inflammation) crowd into these areas. Inflammation develops from the irritation locally, the epithelial cell walls become permeable (allowing transfer of molecules from outside the epithelial cell into the cell interior perhaps leading to changes in the nucleus DNA), alveolitis can develop and lead to fibrosis (scarring) and even tumourigenesis[100, 101].

At this point, we are talking about so-called "pure" soot. However, it should be noted that some of the studies reviewed regarding soot indicate that PAH's were present in soot such as coal dust. This is shown in the Lewis et al review[41] and Table 47 in this addendum (page 72) showed 3 out of the 5 listed PAH's are shown in low levels in the coal dust. These PAH's are thought to affect epithelial cell DNA (the genome) because they have mutagenic effects in the Ames test[101, pp. 25-39].

(ii) Inflammation and Scarring

Finally, as mentioned above it should be reiterated that when the strong inflammatory reaction takes place in the area of lung were overloaded AM are halted, followed by regenerative hyperplasia, then fibrosis (scar) ensues eventually and cancer has been found to arise in scar tissue or near scar areas[92]. The majority of scar cancers are adenocarcinomas but some squamous cell cancer is seen in scar tissue. Unfortunately, scar cancers frequently remain small, while going on to lymphatic and hematologic spread so that distant metastases form before the primary cancer in the lung can be detected on x-ray[92].

B) Mutagenic Fraction and Lung Cellular Effects

(ii) Associated Substances

In diesel exhaust emissions, the mutagenic substances (such as PAH's) frequently enter the lung associated with other gaseous or particulate pollutants (all of them frequently riding on a soot particle), and these other toxic substances can alter the bronchial and alveolar endothelial cell wall. As a result, mutagenic/carcinogenic substances may enter the cell, attack the nuclear DNA and alter the genome causing a pre-cancerous cell or cancer cell[103]. In a study by Patel et al using pork endothelial cells, the fluidity and stability of the lipid membrane of the cell walls was destabilized where the NO_2 remained in contact with the cells all for 120 minutes. It should be noted that with renewed culture medium, the disturbed cell wall integrity recovered 24 hours later. This recovery has not happened in all similar studies but this is

considered to be due to differences in the micronutrients of the culture media[103].

Structural integrity of the cell wall is necessary for normal cellular function internally and to prevent cell wall permeability to noxious substances. The exact consequences of these NO_2-induced cell wall changes remain to be more fully elucidated. However, many studies in the last 12 years have demonstrated that a permeable lipid membrane cell wall allows toxic and/or mutagenic/carcinogenic molecules to enter these cells[1, pp. 368; 268-269].

When squamous cell cancers are examined by the pathologist, metaplastic cells are seen on the periphery with increasing atypia seen in metaplastic cells as the examination moves towards the centre of the cancer. In the centre of the cancer, frank squamous cell carcinoma cells are seen[94].

Not all pathologists subscribe to this hypothesis. Other pathologists refine their description of progressively cancerous cells as (i) hyperplasia of basal cells, (ii) epithelial stratification, (iii) squamous cell metaplasia, and finally (iv) carcinoma *in situ*[92].

These findings are supported by sputum cytology studies. Sputum cells have been followed over ten to 25 years[94] and cells begin as metaplastic, become more dysplastic with increasing atypia and eventually cancerous cells are seen. Figure 42, taken from the twenty-five year sputum cytology study of Saccomano of uranium miners on the Colorado plateau[94]. Thirty-eight cases on Figure 42 were selected because they showed progressive development of metaplasia to carcinoma. Note that oat cell carcinoma appears with squamous cell carcinoma in Figure 42. Note that some patients had double tumours.

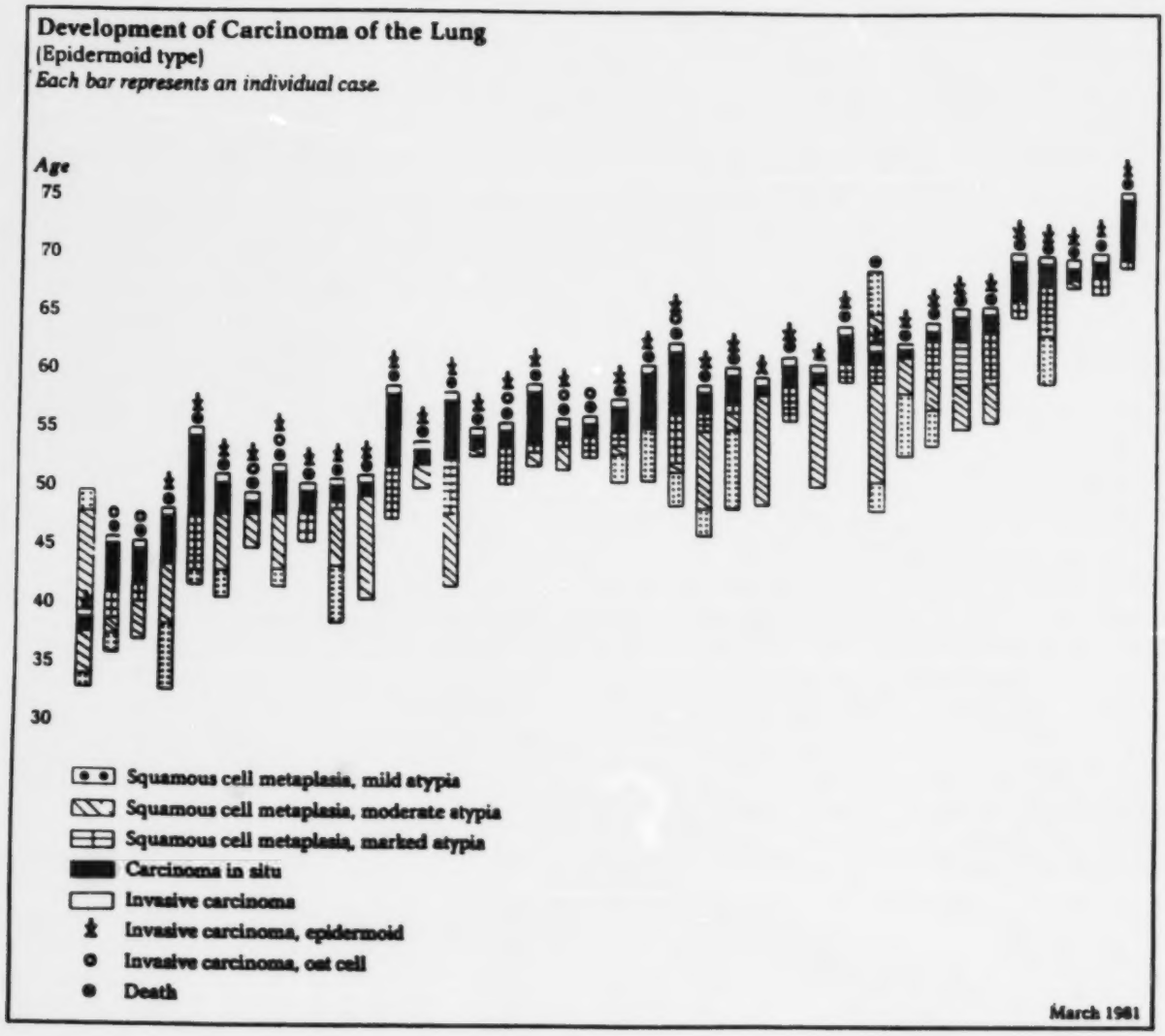


Figure 42

Development of Carcinoma of the Lung

(Source: Reference 94)

These cancers were rare among uranium miners who did not smoke cigarettes and hence the primary carcinogen in uranium miners is considered to be cigarette smoking[94, 96, 97].

In another pathological study in 6 percent of the slides from tumours known to be from patients who were smokers, carcinomas *in situ* that were unsuspected were found in areas distant from the primary cancer which usually was invasive[92]. The cellular development of these remote cancers showed features identical to the main cancer.

Furthermore, sputum metaplastic cells that progressed through moderate and severe atypia also showed increased DNA polyploidy and heteroploidy which has been established as related to carcinogenesis[4, 34, 97].

It should be noted here that some studies have shown that chronic inflammation develops hyperplasia in airway epithelium before metaplasia, dysplasia and carcinoma. In most human studies, inflammation progresses to fibrosis or scarring[92, 97]. As mentioned earlier, cancers have been noted to arise in conjunction to scars.

10.3.4 Development of Human Related Research and Treatment

It was found that the nest of cells with atypia and advanced dysplasia would take up hematoporphyrin. Then these cells, blasted by laser beam at the end of a bronchoscope, would be destroyed and the steady march towards cancer *in situ* would be stopped.

These dysplastic cell nests would be hunted for when atypical cells were found in the sputum of carefully monitored humans.

The problems in this form of research and treatment were many. The only cells that could be blasted by laser had to be in the bronchial tree within reach of a bronchoscope. As we have pointed out, many dysplastic and cancerous areas are on the periphery.

It is difficult to capture appropriate sputum samples from humans on a regular basis. The logistics of trying to get good sputum samples regularly from all potential lung cancer victims are overwhelming.

Consequently, surgical resection with radiation and/or chemotherapy remain major treatments for lung cancer in humans.

10.4 DIESEL EXHAUST HEALTH EFFECTS IN HUMANS

10.4.1 Total Effect

10.4.1.1 Scandanavian Workers Exposed to Total Diesel Exhaust

Ulfvarson et al[104] did detailed studies of several groups of Scandanavian workers exposed to total diesel exhaust emissions.

The first group studied were workers in a bus garage during the months of March and April. In the garage there were gasoline-driven buses as well as large and small diesel buses. There were 3 peak times when diesel particulate accumulated to high levels and ventilation was not considered adequate for the workplace.

Two groups of workers on roll-on, roll-off ferries (ro-ro ships) were studied. One group working on car ferries were studied in June and July. The other groups working as crews on the ro-ro ships were studied in January, February and June.

Both personal and stationary samples of diesel exhaust were taken. The extracts from the filters were not toxic to TA 98 or TA 100 of *S. typhimurium*. However, the extracts were found to be mutagenic but the observed revertants per m^3 of air sample was only 643 for Strain TA-100-S9. The response was lower when metabolic activation was used.

Urine collected from these workers was tested for mutagenicity. However, the majority of samples were non-mutagenic although a few samples displayed values barely at the limits considered to be mutagenic -- greater than 600 revertants/mole of creatinine for *S. typhimurium* or greater than 300 revertants/mole of creatinine for *E. coli*. Interestingly, for the positive urines, all but one samples were submitted by smokers.

For the workers on the ro-ro ships, pulmonary function deteriorated during the work shift. However, after 10 days off work, pulmonary function results compared favourably with the findings before the tested work period began.

Overall, the deterioration of pulmonary function up to 0.4 L on FVC was seen in all work groups during the working shift. There was a decrease of 0.3 L on $FEV_{1\ sec.}$ in all groups. These changes did not impair physical work, did not cause subjective symptoms and returned to normal after 3 days away from diesel exhaust. The FEV% and the MMF remain normal, confusing the nature of the pulmonary deficit. It is suggested by

the authors that the defect is restrictive, not obstructive or a combination of both restrictive and obstructive.

The authors postulate that the NO_2 at levels of 3.6 - 2.9 mg/m^3 can induce pulmonary resistance after exposure for 15 minutes[104].

10.4.2 Pulmonary Function Deficits

10.4.2.1 Obstructive Defect

Since these respiratory deficits have been checked on the animals in many of the recent studies reviewed herein with some confusing results, it seems appropriate to define these deficits here.

When airways are in spasm and therefore the flow rates are lower than normal, the defect is largely seen in reduced factors: $FEV_{1\ sec.}$, FEV%, V_{25}, V_{50}. As well, Airway Resistance (R_{aw}) increases. As the condition advances towards extreme obstruction, Total Lung Volumes become much larger with air trapping, However, the Residual Volume (RV) enlarges first leaving the Vital Capacity (VC or FVC) relatively normal until late in the disease when FVC and VC may become smaller.

10.4.2.2 Restrictive Defect

The deficit is seen when scarring and inflammation make the overall lung tissue stiff. This does two things: (1) the lung volumes become smaller especially the FVC and VC as well as the Total Lung Volume, and (2) with the stiff lung tissue tethering the walls of the airways, the airway diameters become larger so that the flow rates steadily rise until the $FEV_{1\ sec.}$, V_{25} and V_{50} are quite elevated above normal. As well, airway resistance (R_{aw}) is greatly reduced.

10.4.2.3 Mixed Deficits

Naturally, there can be combinations of restrictive and obstructive deficits. For example, one of the purest forms of restrictive disease can be seen in underground hard rock miners who do not smoke and who have pulmonary fibrosis. However, as a large number of miners smoke cigarettes and cigarette smoking causes a marketed obstructive defect, it can be seen that in a cigarette-smoking, hard-rock miner with pulmonary fibrosis there will be both obstructive and restrictive defects. Usually in mild cases of fibrosis, the obstructive defect predominates.

Usually the only way to distinguish between the two defects in one person is by careful assessment of all sections of the pulmonary function test with assessment of the chest x-rays.

10.4.2.4 Research Measurements

Again, researchers have tried to determine why some studies show animals and humans with restriction and some show obstruction.

Bylin et al[105] did studies of human subjects, eight normal and eight asthmatic, exposed to NO_2 during 20 minutes at four different levels (0, 230, 460 and 910 µg/m3). What was most interesting was that the level of NO_2 seemed to play a part in the type of pulmonary deficit that would develop. In the non-asthmatic group, an obstructive defect developed after 20 minutes exposure to 460 µg/m3, but a restrictive defect developed in the same group exposed to NO_2 for 20 minutes at 910 µg/m3.

A number of studies reviewed in this addendum have indicted increased airway resistance[44] (i.e. obstruction), or changes in rats that showed dose-related obstructive and restrictive airways disease[45]. In the latter, the hypothesis was that the changes might be due to hypoxia. However, note was made or the fact that cardiovascular findings in the diesel exhaust exposed rats were consistent with the presence of obstructive lung disease[45].

More studies in animals and humans would confirm the findings of Bylin et al[105]. It may be as these authors hypothesize that different protective mechanisms come into play depending on the level of the noxious substance inhaled.

10.4.3 Diesel Exhaust and Lung Cancer in Railroad Workers

Garshick et al[106] studied a cohort of 55,407 white male railroad workers, 40 to 60 years of age, from the year 1959. These workers had to have started railroad work 10 to 20 years previously and the researchers followed this cohort into 1980. A proportional hazard model was used to calculate the relative risk for cancer as 1.45. Workers with 20 years exposure since 1959 had the highest relative risk. When all results were taken into consideration, it appeared that occupational exposure to diesel exhaust resulted in a small but significant increase in the risk of lung cancer for these railroad workers[106].

10.5 PERSONAL LIFESTYLE OF THE OCCUPATIONALLY-EXPOSED WORKER

10.5.1 Alcohol Use

From the first review in 1979 and continuing in the review of 1984[1], the question of the worker's status with special reference to cigarette smoking was addressed. As lifestyle as a healthy way of living becomes a prominent goal in the life plan of workers in their peak years (25 to 45 years of age), more studies have been done on many aspects of human lifestyle. We review a few special human studies here.

Lange et al[107] studied the effect of alcohol consumption on lung function using the parameters of $FEV_{1\ sec.}$ and FVC. Eleven thousand, one hundred and thirty-five (11,135) men and women from 20 to 90 years of age, stratified according to smoking habits and level of alcohol consumption, were studied for 56 years. Regression analysis demonstrated that significant alcohol consumption accelerated loss in $FEV_{1\ sec.}$ and FVC with the progress of time. A heavy intake of 350 g of alcohol per week had an effect on $FEV_{1\ sec.}$ comparable to the effect of smoking 15 g of tobacco per day[107].

Workers exposed to the obstructive defect as a result of heavy alcohol intake, who are also perhaps reducing the $FEV_{1\ sec.}$ due to diesel exhaust during the work shift[104], and who may also be cigarette smokers, may be setting themselves up for an overwhelming destructive effect on their airways. Respiratory crippling could be a late life result leading to many miserable years of poor quality life before death, even if death comes after 70 years of age.

10.5.2 Cigarette Smoking

10.5.2.1 Impairment of Lung Clearance

In the 1984 review[1], smoking was implicated in paralyzing temporarily at least the cilia that lifts the dust, bacteria and virus-laden mucous out of the airways to the disposal mechanism of the mucociliary escalator.

Cohen et al[108] measured the small amount of a harmless dust(Fe_3O_4) inhaled by three heavy cigarette smokers and nine non-smokers. A magnetic detector was used periodically so that a clearance curve was measured for each subject. The dust clearance in the smokers was much slower than in non-smokers. After a year, 50 percent of the dust remained in the lungs of the smokers whereas in non-smokers only 10% of the dust was retained.

If as implied in several of the animal studies[45, 57, 63] soot or dust plays a role in tumourigenesis, then it would seem that smokers retaining five times the dust that non-smokers retain, are at a much greater risk of tumourigenesis. This risk would exist while the cigarette-smoking miner was underground exposed to diesel exhaust (where 75 - 85% of the soot is respirable) as well as over time while above ground after his shift in the mine is over.

10.5.2.2 Effect of Smoking on Respiratory Symptoms

Tager et al[109] were involved in annual surveys of a cohort from East Boston the U.S.A. At each annual survey, not only was a pulmonary function test done, but a standardized respiratory and smoking questionnaire was completed. There were many variables measured which showed for example that symptomless, non-smoking males did not show an aging effect on the $FEV_{1\ sec.}$ until after age 35. In smoking males, no plateau was seen (to age 35), but aging began showing in the $FEV_{1\ sec.}$ in the early 30's at a slightly more rapid rate than non-smokers.

The conclusion drawn from this study was that the major effect of cigarette smoking on pulmonary function is to start a premature onset of the "normal" rate of aging on respiratory function, and then to increase a more rapid rate of deterioration later on in life. We already know from many other studies that more aged tissues and bodies frequently set the stage for tumourigenesis although this concept needs more exploration[93].

10.5.2.3 The Effect of Cigarette Smoking on Natural Killer Cells - A Major Defence System

A) Natural Killer Cells - their Role

Natural killer cells are a branch or subgroup of lymphocytes which are a major group of white blood cells and these natural killer (NK) cells are in the peripheral blood of normal humans as well as in some animals studied. NK cells as their name suggests, are scavengers searching for tumour or cancer or virus-altered cells which the NK cells then kill[110]. The main interest to date in NK cells has been their ability to destroy malignant cells whereas normal cells seem to be able to resist NK cells.

In animal studies, NK cells were found to prevent distant metastases as well as inhibit the establishment of the primary

cancer or tumour. Consequently, the maintenance of normal state NK cells in the full complement of numbers is a necessity for humans to maintain their internal health and resist all tumours and cancers in the earliest stages of formation[110].

B) The Effect of Smoking

Tollerud et al[111] studied the effect of cigarette smoking on the levels of circulating NK cells. Two hundred and eighty-two (282) subjects from a sample of healthy persons were divided into smokers, ex-smokers and non-smokers. The smokers had a lower percentage ($5.5 \pm 0.3\%$) of NK cells than the subjects who had never smoked ($7.4 \pm 0.4\%$ of lymphoid cells). What is disturbing is that there was also a significantly lower percent ($5.6 \pm 0.4\%$ [$p = 0.002$]) in the ex-smokers including the subjects who had no smoked for more than 20 years, even after controlling for age, sex and total lymphocyte count (higher over all in current smokers).

The above results have indicated that cigarette smoking reduces the number and proportion of NK cells and this defect can last for many years after smoking has discontinued[111].

There now seems to be many ways in which cigarette smoking can alter for the worse the human body's defence mechanisms and leave the human organism more vulnerable to cancer-causing events in its life history.

11. SUMMARY

Tables and figures probably provide the most concise and efficient way to present a summary of the large volume of information presented earlier. For this reason, the data from the seven main research centre's in this field have been summarized, in this format.

Table 76 gives a brief highlight of the different studies. As can be seen, the exposure concentrations ranged between 0.1 and 7.0 mg/m^3 of diesel exhaust parti-culate; the animal species used as subjects included rodents only; and, at this point, the induction of tumours seems to be a function of both exposure concentration and animal species. Table 77 gives details on the exposure conditions used in each of the experiments. Finally, Table 78 summarizes the dose of pollutants administered and the corresponding tumour incidences.

To facilitate the comparison of administered pollutant doses between studies, the concentration units were standardized so that they are the same throughout the tables. Concentrations of benzo(a)pyrene (BaP) and hydrocarbons (HC), as CH_4, were included when available since it was found earlier that PAH's correlated among each other and also with soot. Because these exhaust subfractions have demonstrated a potential for serious health effects these numbers may be used for estimating the potential toxicity of diesel engine exhaust.

Table 76

Long-Term Diesel Health Effects Studies

Laboratory	*Reference*	*Particle Concentration (mg/m^3)*	*Exposure Regimens*	*Animal Species*	*Tumour Induction*
Saitama Medical School	57	2 - 4	16 hrs/wk 24-28 mos	Rats Mice	Negative Positive
Lovelace	42, 59-63	0.35, 3.5 & 7.0	35 hrs/wk 30 mos	Rats	Positive
Battelle	45	0.7, 2.2 & 6.6	90 hrs/wk 24-30 mos	Rats Hamsters	Positive Negative
Fraunhofer Inst.	43	4.0	95 hrs/wk Lifetime	Rats & mice Hamsters	Positive Negative
Japan Automobile Res. Inst.	65	0.1 - 2.0	96 hrs/wk 24 mos	Rats	Negative
General Motors	66	0.25, 0.75 & 1.5	110 hrs/wk 24 mos	Guinea pigs Rats	Negative Negative
Japan Automobile Res. Inst.	65	0.4 - 4	96 hrs/wk 24 mos	Rats	Positive
Japan Res. Inst. of Tuberculosis	46	4.9	56 hrs/wk 24 mos	Rats	Positive

Table 77

Summary of Long-Term Diesel Health Effects Studies: Exposure Conditions

Laboratory	*Engine Type*	*Operation Mode*	*Conc. (mg/m^3)*	*Exposure Regimen*	*Exposure Period*
Saitama	Yanmar 269 cc	Idle	2 - 4	4 hours/day 4 days/week	24 - 28 months
Lovelace	Oldsmobile 5.7 L	FTP	0.35, 3.5, 7.0	7 hours/day 5 days/week	30 months
Battelle	VW 1.5 L	FTP	0.7, 2.2, 6.6	16 hours/day 5 days/week	24 - 30 months
Fraunhofer	VW 1.6 L	FTP	4.0	19 hours/day 5 days/week	Lifetime
JARI	1.8 L, IDI, 4 cycle	1700 rpm, 36% load, 50 kph	0.1 - 2.0	16 hours/day 6 days/week	24 months
GMRC	Oldsmobile 5.7 L	65 kph	0.25, 0.75, 1.5	20 hours/day 5.5 days/wk	24 months
JARI	11 L V6, DI	1200 rpm, 50% load, 85 kpm	0.4 - 4	16 hours/day 6 days/week	24 months
JRIT	2369 cc	1000 rpm 80% load	4.9	8 hours/day 7 days/week	24 months

Table 78

Long-Term Diesel Health Effects Studies: Administered Dose

Laboratory	Exposure Regimens	Particle Conc. (mg/m^3)	% Extractables	BaP* Production Rate (µg/h)	Gaseous HC* Production Rate (g/h)	Animal Species	Excess Tumours (%)	Excess Malignant Tumours (%)
Saitama	16 hrs/wk 24-28 mos	2 - 4	---	0.47	---	Rats Mice Mice	7.6 6.0 8.0	0.0 2.8 2.6
Lovelace	35 hrs/wk 30 mos	7.0	25.1	74	6.9	Rats Rats	14.7 3.2	8.4 0
Battelle	90 hrs/wk 24-30 mos	6.6 2.2	14.5 ---	20.7 146	6.0 ---	Rats Rats	37.0 8.3	--- ---
Fraunhofer	95 hrs/wk Lifetime	4.0	---	19.3	5.7	Hamsters Mice Rats	0 19 17.8	0 14.6 9.5
JARI	96 hrs/wk 24 mos	2.3 1.1	--- ---	8.4 ---	2.1 ---	Rats Rats	0 0.8	0 0.8
GMRC	110 hrs/wk 24 mos	1.5 0.75	39.3 ---	30 ---	--- ---	Rats Guinea pigs	--- ---	3.3 10.0
JARI	96 hrs/wk 24 mos	3.7 1.8	--- ---	6.7 ---	3.5 ---	Rats Rats	5.7 2.5	5.7 2.5
JRIT	56 hrs/wk 24 mos	4.9	---	---	---	Rats	37.9	26.3

(*) HC = Hydrocarbons; BaP = Benzo(a)pyrene

Tumour incidences were dose dependent at higher concentrations and were statistically significantly higher than controls with particulate concentrations of at least $2.2mg/m^3$. These changes were observed mostly in rats but also in mice, but never to a significant extent in other species. Lower soot levels produced less serious changes in pulmonary, histological and haematological parameters. As Vostal[6] observed, tumours were found mainly in females and mostly in animals older than two years.

Figures 43 and 44 illustrate the relationship between particulate concentration and either excess lung tumours or excess malignant lung tumours, respectively. Excess tumours are those observed after adjustment for the tumours that occured in control animals. The trend indicates a dose-response relationship. The scattering of data points at low particle concentrations prevents the confirmation or repudiation of the possibilty of a threshold for tumour induction.

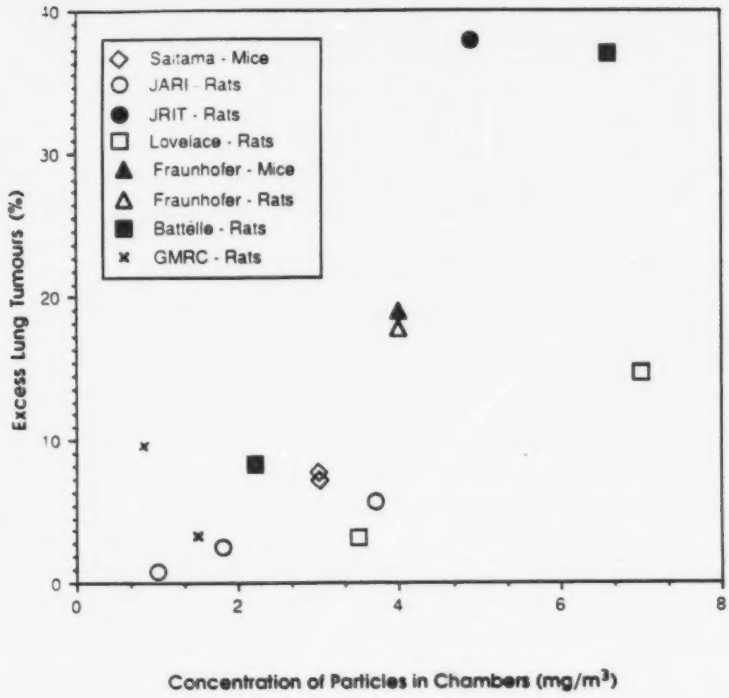


Figure 43

Relationship Between Particulate Exposure Concentration and the Number of Excess Lung Tumours

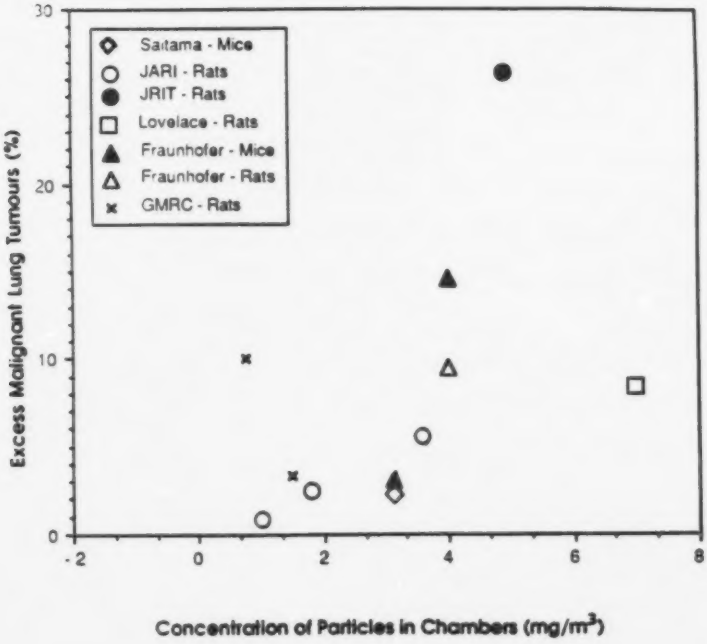


Figure 44

Relationship Between Particulate Exposure Concentration and the Number of Excess Malignant Lung Tumours

Figure 45 (a plotting of excess lung tumours against exposure per week) and Figure 46 (excess lung tumours against percent load), do not present any concrete relationship. As a consequence, it can be concluded that the engine load (i.e. particulate character) and exposure duration do not seem to alter the threshold effect observed with particle/mutagen concentration.

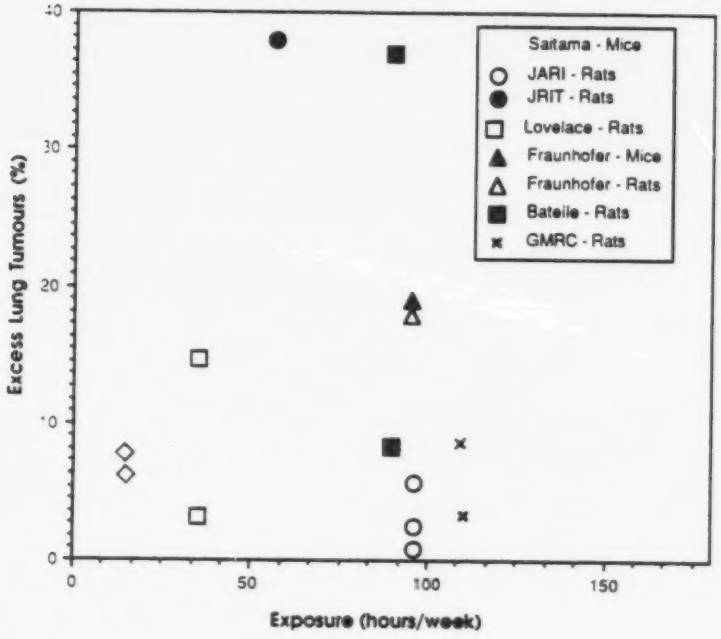


Figure 45

Effect of Length of Exposure Period on the Number of Excess Lung Tumours

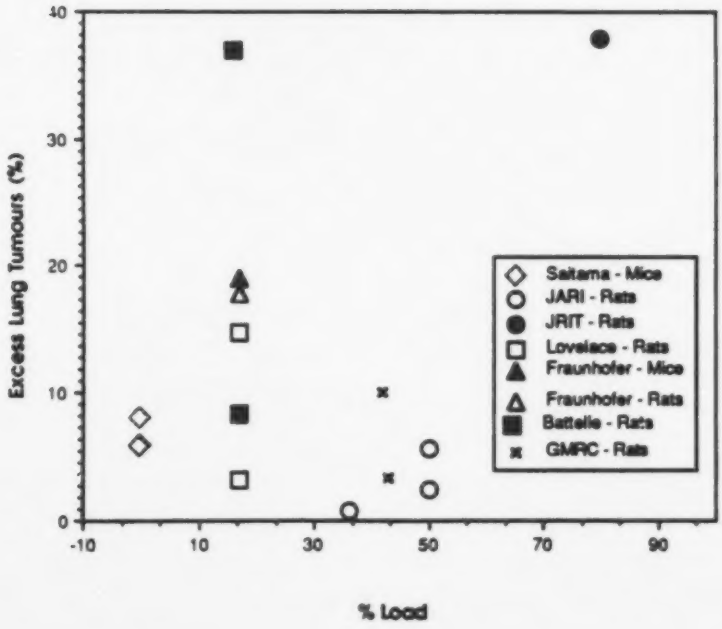


Figure 46

Effect of Percent Engine Load on the Number of Excess Lung Tumours

Finally, Figure 47 presents excess lung tumours against mutagen concentration in the chambers. As in Figure 43 and in Figure 44, a distinct dose relationship emerges.

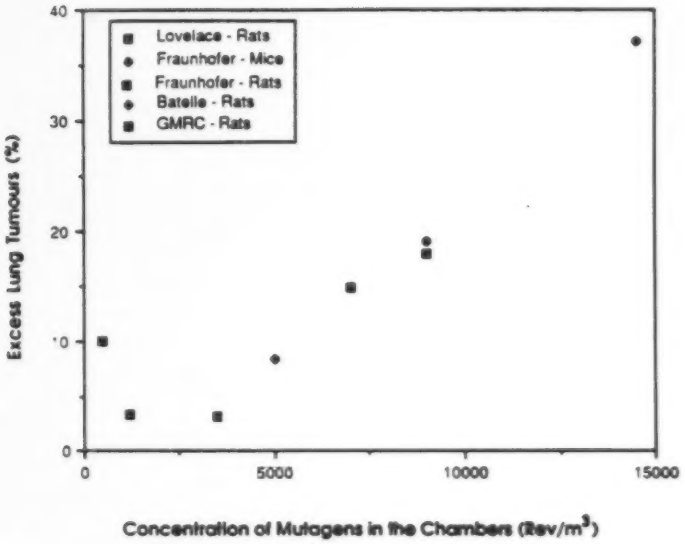


Figure 47

Relationship Between Mutagen Concentration in Exposure Chambers and the Number of Excess Lung Tumours

12. CONCLUSIONS

Unarguably, a risk-free environment is the most desirable of situations from a health and safety standpoint. However, a totally safe or risk free society is not attainable. The obvious need to balance costs and benefits when new equipment is designed or when regulations are drafted have resulted in the creation of such tools as Threshold Limit Values (TLV's) and the Air Quality Index (AQI) equation.

As Muir and Verma[79] mentioned, the present lack of irrefutable demonstrations of lethal health risk is not a suitable excuse for inaction. It would be futile to wait for adequate long-term epidemiological data that proves the toxicity of diesel engine exhaust if, at the time such information is available, it is too late to help affected individuals.

The Air Quality Index (AQI) formulae that were proposed in earlier editions of this report still retain their original purpose: to assist in determining whether or not the pollutant levels in mine atmospheres represent a health risk to exposed workers.

The magnitude of the different TLV's used in the AQI formulae has not been revised in the last four years. However, the Occupational Safety and Health Administration (OSHA) is proposing more restrictive standards, in a few cases, at the time this report is being completed. Specifically, Table 80 reflects the changes that are under review (Table 80).

As shown in the introduction, the AQI equations have the following format:

$$\textit{AQI (particulate):}\quad \frac{[RCD]}{TLV_{RCD}} + \left[\frac{[SO_2]}{TLV_{SO_2}} + \frac{[RCD]}{TLV_{RCD}}\right] + \left[\frac{[NO_2]}{TLV_{NO_2}} + \frac{[RCD]}{TLV_{RCD}}\right]$$

$$\textit{AQI (gas):}\quad \frac{[CO]}{TLV_{CO}} + \frac{[NO]}{TLV_{NO}} + \frac{[NO_2]}{TLV_{NO_2}}$$

It was recommended that the AQI (particulate) value not exceed 2.0 and that no sigle component exceed its TLV, as dictated by current ACGIH standards. Similarly, the AQI (gas) was not to exceed 1.0 and no individual component, its TLV.

In the last four years, the assumptions underlying the last statement have met some opposition. It is said that the addition of the carbon monoxide (CO), nitric oxide (NO) and nitrogen dioxide (NO_2) terms in the AQI (gas) formula implies that the health effects of these compounds is additive. This results in an incorrect application of the Rule for Mixtures because the TLV's for CO and NO have been set to avoid reduction of the oxygen carrying capacity of hemoglobin, whereas the TLV for NO_2 has been set to avoid lung injury.

Table 80

ACGIH or NIOSH Limits that OSHA Considers Proposing

Chemical Name	*ACGIH TLV**		*NIOSH REL***	
	TWA	*STEL*	*TWA*	*Ceiling*
Carbon Monoxide	50 ppm	400 ppm	35 ppm	200 ppm (NDT)
Nitric Oxide	25 ppm	Not considering any change	Not considering any change	Not considering any change
Nitrogen Dioxide	3 ppm	5 ppm	---	1 ppm (15 min.)
Sulphur Dioxide	2 ppm	5 ppm	0.5 ppm	---
RCD <5% quartz	2 mg/m^3	---	---	---
RCD >5% quartz	0.1 mg/m^3	---	---	---

Note: Underlined values indicate the limits being proposed by OSHA
NDT no determined time; RCD = respirable coal dust

(*) The American Conference of Governmental Industrial Hygienists (ACGIH) TWA-TLV is for an 8-hour exposure; its Short Term Exposure Limit (STEL's) are 15 minute limits not to be exceeded more than 4 times per day with a minimum of 60 minutes between successive STEL exposure; and its ceilings are peaks not to be exceeded for any period of time

(**) National Institute for Occupational Safety and Health (NIOSH) TWA limits are for 10-hour exposures unless otherwise specified, and its ceilings are peaks not be exceeded for any period of time unless a duration is specified in parentheses.

The AQI equation was intended to evaluate the potential toxicity of air in a mine or other environment. Such a purpose dictated that the different components of toxicity should be totalled so as to obtain an estimate of the overall health risk that the environment represents. The separation of the NO_2 term from CO and NO should only take place in the interpretation of the AQI value and not in the structure of the expression itself. If the offending term was moved to a separate equation, the toxicity estimate then obtained would be specific for certain organs. The AQI (gas) would not give the desired overall health risk. Under this light the equation is believed to be logically sound and is left untouched. Also, as before, no constituent should be permitted to rise above its TLV and the value of the AQI (gas) should not exceed 1.0.

The addition of a coefficient in front of the NO_2 term of the AQI-gas formula would be an eventual improvement of the expression. Such a number would modify the weight of the NO_2 term so that it is more representative of this gas' toxicity relative to that of NO and CO. Unfortunately, the data presently available does not allow the determination of how much more or less of a risk NO_2 represents in comparison with CO and NO. For this reason, the addition of a coefficient at this time would be arbitrary and possibly

misleading. Consequently, it is recommended that the AQI(gas) equation not be revised at this time.

The original equation was broken down to the two formulae presented above to differentiate the gas effects from the particulate (RCD) effects. This development was not considered of primordial importance by the Collaborative Diesel Research Advisory Panel (CDRAP)[85] which has continued to use the single equation for consistency. In their opinion, both equation systems give virtually identical results from the standpoint of fresh air dilution ratio. However, the use of the expression in its updated form is preferable since it corrected some of the inaccuracies in the original formula.

The previous sections have shown that, although tumours may be encountered at particulate levels below 2 mg/m^3, their incidence becomes statistically significant at this concentration. The Japan Automobile Research Institute[65] showed that thirty months of exposure to 2.0 mg/m^3 of diesel exhaust particulate resulted in considerable lung accumulation. Also, findings from the National Institute for Safety and Health (NIOSH)[40] demonstrated a decrease in macrophage activity and a consequent decrease in lung clearance after exposure to 2 mg/m^3 of exhaust.

Knowing that the TLV for RCD is presently at 2.0 mg/m^3, these results are cause for concern. Unfortunately, little data are available at low soot concentrations. What is more, the published information is far from unanimous: the summary figures presented earlier testify of the results lack of resolution at low RCD levels.

Despite these problems, toxicity data suggests that it would be prudent to lower the TLV for RCD. In considering more restrictive values, one must allow for a certain margin of safety while keeping in mind the limits of current exhaust purification equipment and the costs of achieving the proposed emissions levels. It is recommended that the new TLV for diesel exhaust particulate be lowered to 0.5 mg/m^3.

The minutes of a recent meeting of the Ad Hoc Committee on Diesel Emissions reveal that such a level is attainable. They provided the following calculations:

> *"If the soot concentration in undiluted unfiltered exhaust is 70 mg/m^3 (a high, full load/speed value for a Deutz F6L912W engine), and it is diluted 70 times (typical in underground workings) by the air supplied, the diluted value is 1.0 mg/m^3. If a 90% efficiency filter is installed, the undiluted soot emitted is 7 mg/m^3 and when diluted 70 times, the ambient concentration is 0.1 mg/m^3."*

The proposed 75% reduction in this TLV is, therefore, not excessive.

Data available on the health effects of soot concentrations below 1.0 mg/m^3 show that the 0.5 mg/m^3 would allow for a margin of safety:

- Griffis et al[36] showed that, in rats, particulates accumulate to the same negligible extent at either 0.15 or 0.94 mg/m^3 once lung burden has been normalized for exposure concentration.

- According to Ishinishi et al[65], adenoma and carcinoma incidences are both comparable to those seen in controls when rats are exposed to 0.4 and 1.0 mg/m^3 of exhaust.
- Mauderly et al[63] reports similar findings for rats exposed to a particulate level of the order of 0.35 mg/m^3.
- and, Brightwell et al[45] obtained statistically insignificant tumour incidences in rats and hamsters at 0.7 mg/m^3.

In light of these findings, the reduction of the TLV to the 0.2 mg/m^3 level mentioned in the Ad Hoc committee minutes does not appear necessary. Experimental rats, it should be emphasized, are the animals in which the above exposure levels produced no tumours. Since these rodents are generally more sensitive to diesel exhaust than other laboratory animals, using these results is in agreement with the EPA's policy of choosing the most sensitive species tested when selecting data for extrapolations. Once again, as Perera[83] said:

> *"This is not only consistent with the aim of being health protective, but is based on evidence that humans are, in most cases, as sensitive or more sensitive than experimental animals."*

The reduction in the TLV for RCD does not require a comparable reduction in the maximum allowable value of the AQI(particulate) equation: the denominator and quotient of each of the affected terms will change proportionally. Therefore, the AQI(part) limit value can remain at 2.0.

The epidemiological evidence accumulating since 1984 supports the contention that occupational exposure to diesel exhaust is associated with an increased risk of lung cancer[88 - 91]. Nevertheless, some experts can only agree on the rather weak statement:

> *"Taking all the information together, it would appear appropriate to conclude that whole diesel exhaust is a probable human carcinogen"*[86].

In contrast, some others have a more prudent and conservative public health approach[113]:

> *"Recent animal studies in rats and mice confirm an association between the induction of cancer and exposure to whole diesel exhaust. The lung is the primary site identified with carcinogenic or tumourigenic responses following inhalation exposures. Limited epidemiologic evidence suggests an association between occupational exposure to diesel engine emissions and lung cancer. The consistency of these toxicologic and epidemiologic findings suggests that a potential occupational carcinogenic hazard exists in human exposure to diesel exhaust. tumour induction is associated with diesel exhaust particulates. Limited evidence indicates that the gaseous fraction of diesel exhaust may be carcinogenic, as well."*

Again, it is the authors' opinion that the AQI formulae can assist in determining acceptable pollutant levels in dieselized mines. Their use should provide a reasonable certainty of no significant health effects from the use of heavy duty diesel engines underground.

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